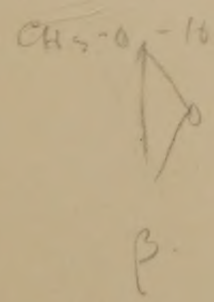
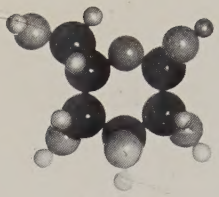

CH3-O
H
OCH3
β
α glucoside

THE CONSTITUTION OF SUGARS

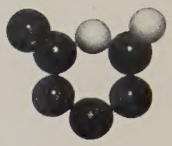
DEDICATED

TO

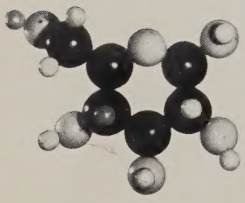
MY WIFE



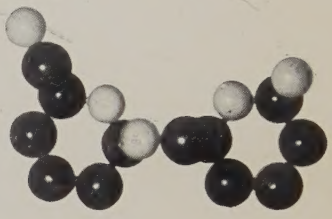
MODEL OF β-GLUCOSE



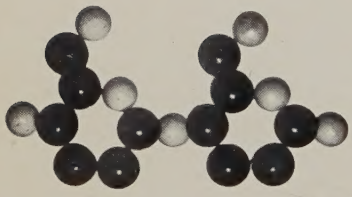
SKELETON MODEL OF
β-GLUCOSE



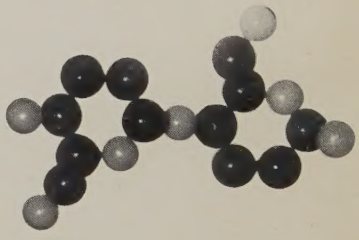
STRAINLESS MODEL FOR
β-GLUCOSE (*See Fig. III, p. 91*)



SKELETON MODEL OF
GENTIOBIOSE



SKELETON MODEL OF MALTOSE



SKELETON MODEL OF CELLOBIOSE

ATOM MODELS OF SUGARS

THE CONSTITUTION OF SUGARS

BY

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PREFACE

Many friends, and especially those working in other fields of chemistry, have suggested that I should write a book of this kind. In it I have endeavoured to present a clear and concise account of the experimental work on which the new constitutional formulæ of sugars are founded.

The fulfilment of this general wish has been facilitated by the use of the manuscripts I had already prepared on this subject, in the spring of 1928, for a course of six lectures at the University of Bâle, and for other lectures which I gave at Zürich, Heidelberg, Neuchâtel, and Mulhouse.

I am conscious that, in so far as my name is associated with the researches from Birmingham, and formerly from Newcastle, the greater share of credit should be given to my colleagues and pupils, since it is mainly by their experimental skill and resource that these results have been achieved.

A special acknowledgment is made in this connexion to my colleague and former pupil, Dr. E. L. Hirst, whose personal share in this work cannot be valued too highly ; and to Dr. H. D. K. Drew, whose critical faculty and philosophic outlook have been of the greatest service. I am also grateful to both of them for reading through the proofs of this book.

Much of the development herein outlined has been due to the study of methylated sugars, and a tribute is here paid to the memory of Thomas Purdie, whose first two papers on this subject were published just twenty-five years ago. His friendship, which I enjoyed during the closing years of his life, inspired in each and all a response which was warmer than personal esteem.

W. N. HAWORTH.

The University of Birmingham,
December, 1928

CONTENTS

PLATES

The abbreviated references used in this book are the same as those adopted in the Journal of the Chemical Society.

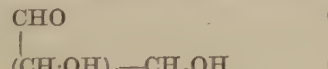
THE CONSTITUTION OF SUGARS

CHAPTER I

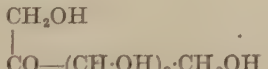
INTRODUCTORY REVIEW OF THE REACTIONS AND THE OLDER FORMULÆ OF SUGARS

The constitution of the carbohydrates must ultimately rest upon the ascertained structure of the simplest units which are represented by the sugars. In the study of the sugars, which involves an inquiry into the properties of their numerous derivatives, problems are encountered which are not merely structural but stereochemical. It may indeed be said that there is no group of organic compounds in which stereoisomerism has so significant a place. For this reason the use of atom models is essential even to students already familiar with the subject. The models are preferably made by constructing spheres of a magnitude proportionate to those which have been deduced from X-ray data for carbon and oxygen atoms, and the spheres should be assembled by the use of hidden links or valency bonds inclined at an angle of 109°28′ to each other. Some of the misleading interpretations which have delayed development in the carbohydrate field might have been avoided by more frequent recourse to models as a means of visualizing structural and stereochemical formulæ.

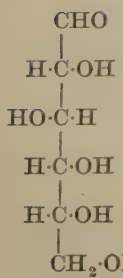
Reference to the conventional aldehyde and ketone formulæ which were assigned to the sugars will sufficiently emphasize their inadequacy as a complete method of representation :



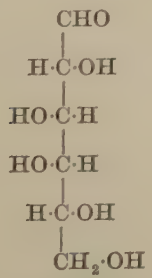
Aldose



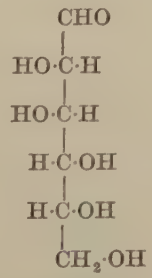
Ketose



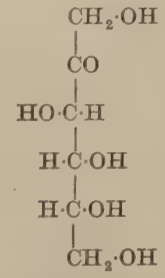
d-Glucose



d-Galactose

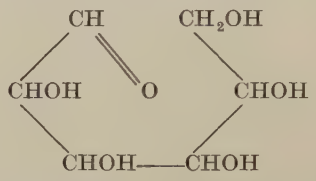


d-Mannose



d-Fructose

Here is furnished a mental picture of the distribution of the hydroxyl groups in a sugar, and indeed it is essential to adopt these projection formulæ at the outset. It must be remembered, however, that these projection formulæ are founded on the convention that in the model the addenda attached to the carbon atoms of the central chain are above the plane of the paper. This result can only be achieved by theoretically uncoiling the carbon chain from its spiral form, inasmuch as the tetrahedral angle between adjoining carbon valencies requires that the atom model of a sugar should resemble more the nature of a ring than an open chain. A point which will be further developed is that the properties of the simple sugars are dependent upon the proximity of the supposed aldehyde group in such a formula to the hydroxyls attached to the fourth or fifth carbon atom. The convention of writing plane formulæ obscures in many respects this important feature although it is helpful in others.



Such considerations find an immediate illustration in the formulæ ascribed initially by Fischer [1] to the methylglucosides, etc. All the reducing sugars condense with methyl alcohol in the presence of small concentrations of hydrogen chloride, a reaction which leads in the case of glucose to two definite crystalline products.

$$\text{Glucose} \xrightarrow[\text{digested at 65° under reflux}]{\text{MeOH} + 0{\cdot}5\%\ \text{HCl}} \begin{matrix}\text{Normal}\\ \alpha\text{-Methylglucoside}\\ +\\ \beta\text{-Methylglucoside}\end{matrix}$$

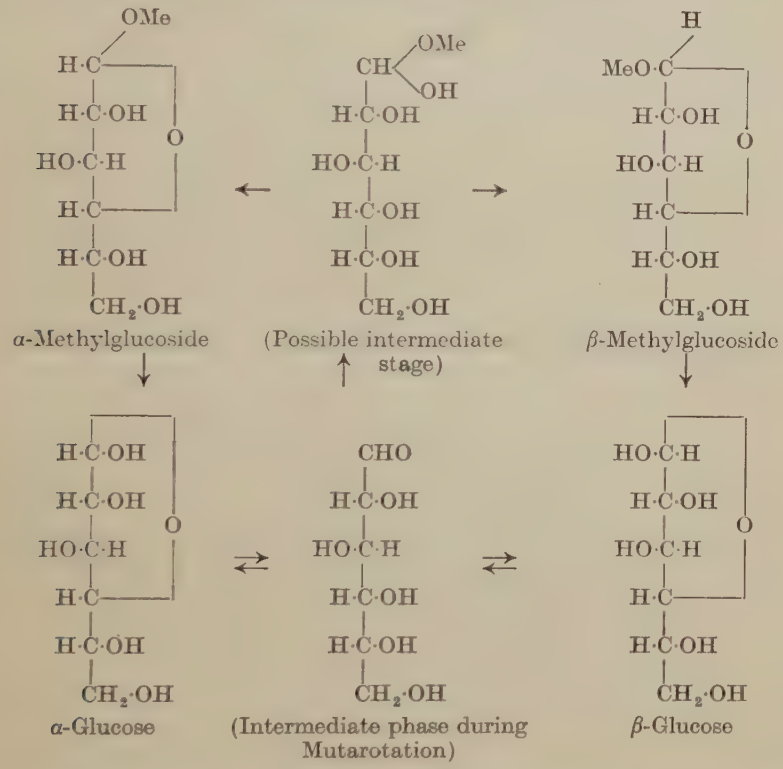
Since the effect of this reaction is to increase the molecular formula of glucose from $C_6H_{12}O_6$ to $C_7H_{14}O_6$, it is clear that some kind of condensation, as distinct from addition of methyl alcohol, has occurred. Whatever may be the structure, whether cyclic or open-chain, of the original glucose, there is no doubt that for the two methylglucosides a cyclic formula is demanded. The reaction is indeed equivalent to acetal formation, analogous to the familiar reaction between an aldehyde and an alcohol:

$$\text{R·CHO} + 2\text{MeOH} \rightarrow \text{R·CH(OMe)}_2 + \text{H}_2\text{O}.$$

But in the case of a sugar one methoxy residue only is introduced, the remaining ether grouping being satisfied by the union with a

[1] *Ber.*, 1893, **26**, 2400. Compare E. Fischer, *Untersuchungen über Kohlenhydrate und Fermente*, 1909, p. 88.

hydroxyl group intermediately situated in the sugar chain. Moreover, the properties of the methylglucosides correspond almost exactly with those of acetals. They are comparatively stable to dilute alkalies and are easily hydrolyzed, on boiling with dilute acids, to give the parent sugar, whether aldose or ketose. The formulæ assigned by Fischer to the methylglucosides, as given below, represent them as heterocyclic compounds consisting of five-atom rings.



These formulæ have the merit of representing and accounting for the existence of two stereochemical forms of glucoside which are structurally identical, differing only in the disposition of the methoxy residue which is directed to the right in the α-glucoside and to the left in the β-glucoside. The occurrence of two forms was satisfactorily explained by the adoption of the cyclic structure. It is, however, evident that the selection of the five-atom ring, in preference to a three- or four- or six-atom ring, had no experimental basis beyond that of mere analogy with the lactones, which will be discussed subsequently. Additional significance was given to the cyclic formulæ of the glucosides by the later discovery of the existence of free glucose in α- and β-stereoisomeric forms, having $[\alpha]_D + 110°$ and $+ 17{\cdot}5°$ respectively.

The inference was drawn by Simon that the disclosure of two forms of glucose, each being capable of mutarotation or interconversion into the other in aqueous solution, presented a *prima facie* case for the representation of the two forms of crystalline glucose by cyclic formulæ also. It was left to E. F. Armstrong [1] to demonstrate that hydrolysis of α-methyl-glucoside led to the initial formation of α-glucose, and the hydrolysis of β-methylglucoside to the formation of β-glucose, and hence the relationship expressed above in the second line of formulæ appeared to be established. The aldehyde form of glucose is represented in this scheme as an intermediate phase only between two cyclic α- and β-forms of glucose which exist side by side in aqueous solution. For the interpretation of the behaviour of sugars during mutarotation, chemists are indebted to the work of T. M. Lowry.[2]

A characteristic reaction of aldoses is illustrated by the ease with which, for example, glucose passes on oxidation with bromine water into gluconic acid, and then spontaneously or by heating into γ-gluconolactone. The lactone is given the formula of a five-atom ring structure, which is now quite definitely established. It was one of the many achievements of Fischer [3] to effect the reconversion of such a lactone to the parent sugar by reduction; for instance, *d*-gluconolactone is reduced by sodium amalgam in the presence of dilute sulphuric acid to *d*-glucose. Doubtless the evidence of this simple change was considered sufficient to correlate the ring structure of glucose with that of its corresponding lactone.

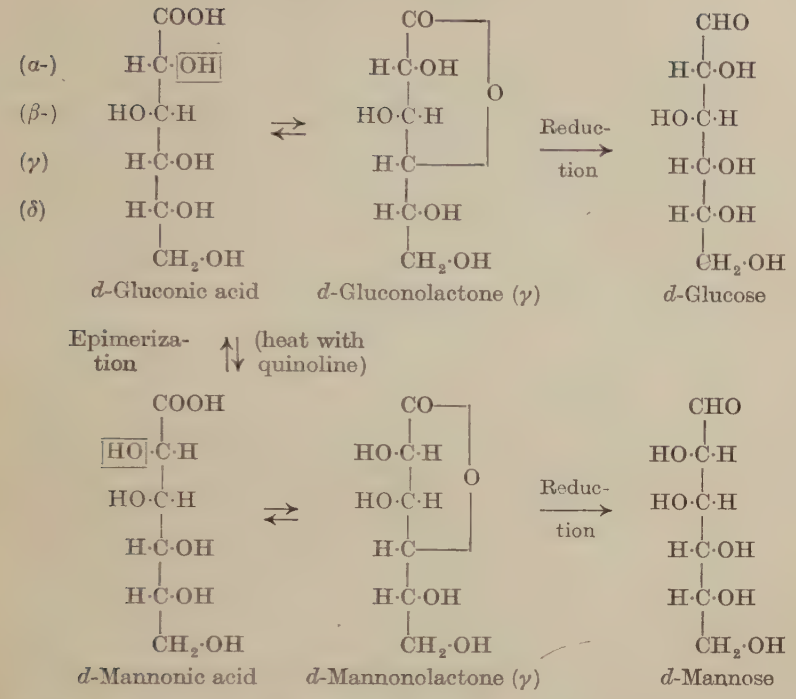
The inter-relationship of glucose and mannose was illustrated [4] by the sequence of changes indicated in the formulæ on the next page:

[1] *J.*, 1903, **83**, 1305.

[2] Summarized in *Z. physikal Chem.*, 1927, **130**, 125.

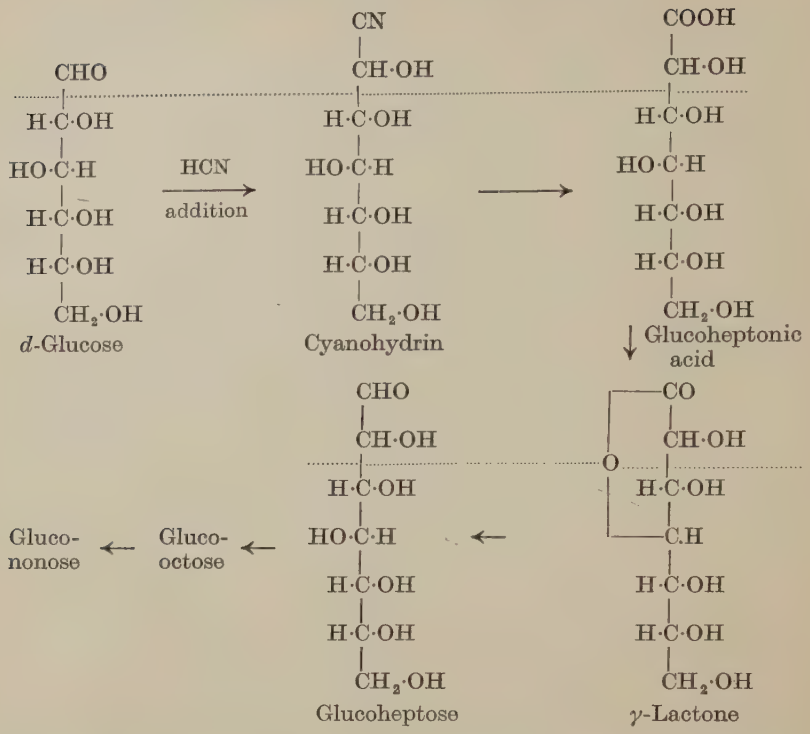
[3] Consult *Untersuchungen über Kohlenhydrate und Fermente* (*pub.* Berlin, 1909) for all references to Emil Fischer.

[4] E. Fischer (*loc. cit.*).

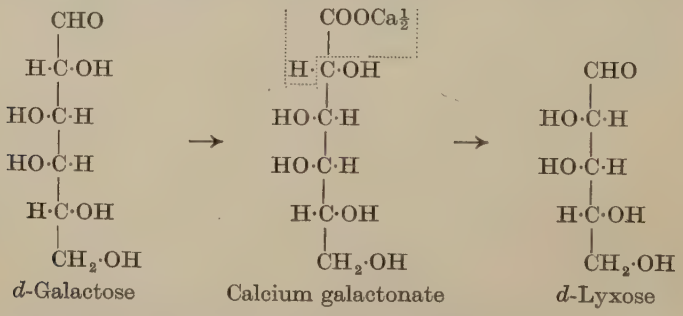


The heating of gluconic acid with quinoline or with aqueous pyridine effected the inversion of the groups at the α- or second carbon position, a change (known as epimerization) which leads to the formation of *d*-mannonic acid, and therefore to the γ-mannonolactone, and finally, by reduction, to mannose. An analogous behaviour is characteristic of all the aldose series of sugars. Other representative reactions may now be summarized. Thus, glucose combines additively with hydrogen cyanide to give a cyanohydrin, which passes by hydrolysis to glucoheptonic acid.[1] This again yields a γ-lactone and, by reduction, gives rise to the next higher sugar in the series, namely glucoheptose. By repeating this reaction one can continue the ascent of the series to gluco-octose and to gluconononose, etc.

[1] Maquenne, *Compt. rend.*, 1888, **106**, 286; Kiliani, *Ber.*, 1888, **21**, 915; 889, **22**, 521; E. Fischer (*loc. cit.*).



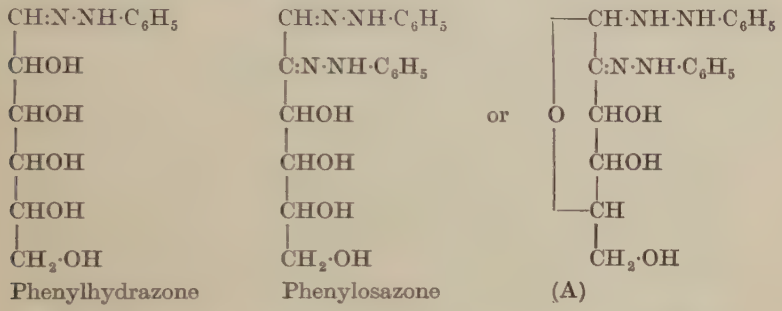
A corresponding descent of the sugar series can be effected in several ways. One of the most satisfactory is the oxidation of the calcium salt [1] of, for example, gluconic acid, with hydrogen peroxide in the presence of a trace of ferric iron. This leads to the elimination of the first carbon atom of the glucose chain with the formation of *d*-arabinose. It is by the use of such experimental methods that certain rare sugars which do not occur in nature have been prepared; for example, a similar procedure to that just described leads by descent of the series from galactose to lyxose.



Among the most useful of all the reactions which sugars undergo

[1] Ruff and Ollendorf, *Ber.*, 1900, **33**, 1798.

is that towards phenylhydrazine and related bases.[1] Glucose and other sugars form crystalline phenylhydrazones which, however, react with a further quantity of phenylhydrazine to give the characteristic crystalline derivatives known as the phenylosazones of the sugars. This reaction involves the attachment of two phenylhydrazine residues to the sugar chain, a result which can only have been achieved by the utilization of some of the phenylhydrazine to oxidize a secondary alcohol group to a keto group.



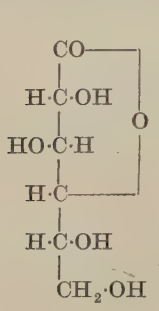
The final word on the structure of phenylosazones has probably not been given. These compounds mutarotate readily in aqueous alcohol or in pyridine as indeed do the phenylhydrazones. It is not impossible to explain these properties on the assumption that at least one phenylhydrazine residue is attached as a phenylhydrazide group in a cyclic sugar as indicated by formula (A) above.

[1] E. Fischer (*loc. cit.*).

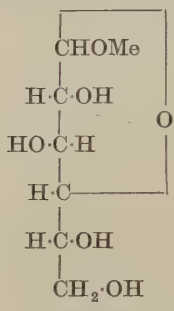
CHAPTER II

SOME PROPERTIES OF LACTONES CONSIDERED IN RELATION TO RING STRUCTURE

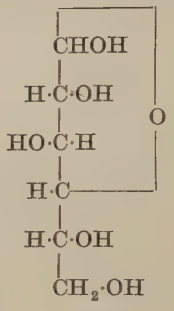
The circumstance that lactones derived from sugars were formulated as five-atom ring compounds was held to be sufficient reason for assuming a like constitution for α- and β-methylglucosides.



γ-Gluconolactone



α- and β-Methyl-glucosides



Glucose

During more than forty years this analogy had sufficed as the chief or only reason for the adoption of Fischer's glucoside formula or for its extension to glucose itself. When it is realized, however, that an experimental basis for the formulation of γ-gluconolactone was wanting, it will be conceded that constitutional formulæ in the carbohydrate group rested upon an insecure basis.

An endeavour was made to meet this difficulty by the theoretical considerations advanced by C. S. Hudson.[1] He reviewed the physical properties of some 24 crystalline lactones derived from sugars and reached a conclusion which is known as "Hudson's lactone rule." Having regard to the projection formulæ applied to the monocarboxylic acids derived from and related to simple aldoses, he deduced by the method of trial that in the cases of all the 24 lactones the γ- or five-atom ring structure was correctly assigned. Thus, adopting this structure, he observed that where the oxide-ring of the lactone was formed by engaging a hydroxyl group on the right of the carbon chain, the optical rotation was enhanced in the dextro sense. Conversely, where the lactone ring was formed through the participation of a hydroxyl group on the left of the projection formula of the monobasic

[1] *J. Amer. Chem. Soc.*, 1910, **32**, 338.

acid, the optical rotation of that lactone was increased in the lævo direction.

Assuming the γ-lactone structure to hold in all the cases of the 24 lactones under review, he was therefore able to formulate the "lactone rule," which broadly states that where the formation of the oxide-ring engages a hydroxyl which is situated on the right of the projection formula, the conditions favour dextrorotation; and the contrary conditions, where the participating hydroxyl group is on the left, favour lævorotation. This appeared to hold without any exception. On the other hand, if any other ring than the five-atom ring be assigned uniformly to these lactones, there appeared to be no such relationship between optical rotation and configuration. Mathematical calculations of probability pointed therefore with a large measure of certainty to the recognition of these lactones as γ-lactones, that is cyclic compounds having the five-atom ring structure. For the sake of interest and reference a number of the known γ-lactones are formulated on pages 15 and 16, along with the sign and numerical value of their specific rotations.

It will be seen that where the oxide ring is on the right the sign is dextro, and on the left, lævo.

More recent work on the experimental side served to confirm this simple and helpful speculation, which was published by Hudson in 1910, but it might have been difficult in the year 1914 to accommodate into this generalization the new β-glucono- and β-mannono-lactones isolated by J. U. Nef.[1] If the formula of a γ-lactone be compared with the hitherto accepted cyclic formulæ of α- and β-sugars to which the lactone gives rise on reduction, the suggestion emerges that a relationship between ring configuration and the sign of rotation, similar to that of the lactone rule, might also hold for the sugars themselves. In the latter case, however, an asymmetric centre exists at the first carbon atom of the chain where the $>$CHOH group replaces the CO group in the lactone. On this view the presence and configuration of H and OH in α- and β-glucose may be wholly responsible for the alteration in specific rotation which accompanies the change from lactone to sugar. Such, indeed, may be expected only if two conditions hold: first, that the five-atom ring structure of the lactone is unimpaired in the change to the derived sugar; and secondly, that the mean of the specific rotation values of α- and β-glucose can be taken for the purpose of comparison with the rotation of the lactone. That the second of these hypotheses may hold only in an approximate sense is clear on the basis of van't Hoff's general principle of optical superposition. Thus, if one divides the sugar molecule into two parts C_1, being the reducing group, can be regarded as $+a$ in α-glucose

[1] *Annalen*, 1914, **403**, 204.

and $-a$ in β-glucose, whilst the rest of the molecule will be considered as b. The rotation of α-glucose will then correspond with $+a+b=+110°$ and for β-glucose, $-a+b=+18°$. Adding the equations the value $2b$ is given by $+128°$, and therefore the value of b is 64° in the sugar. This neglects entirely, however, the contribution which the group C_1 makes to the total rotation of b, if the latter value is to be used both for the lactones and the sugars. It cannot be held that the rotation contributed by $\pm a$ is without effect on b. But assuming that this contribution will be relatively small so that it does not dominate the total rotatory effect at the centres C_1 to C_4, then the expectation is that some generalization connecting configuration and rotation might apply also to the sugars. That this is not even approximately true, and is, indeed, widely divergent from the real facts of the case was pointed out by Fischer [1] in comparing the members of the mannose series. He remarks as follows: "The optical behaviour of the new compounds is worthy of note. Apparently the rotatory power changes in an irregular way from right to left and inversely, as the following classification shows:

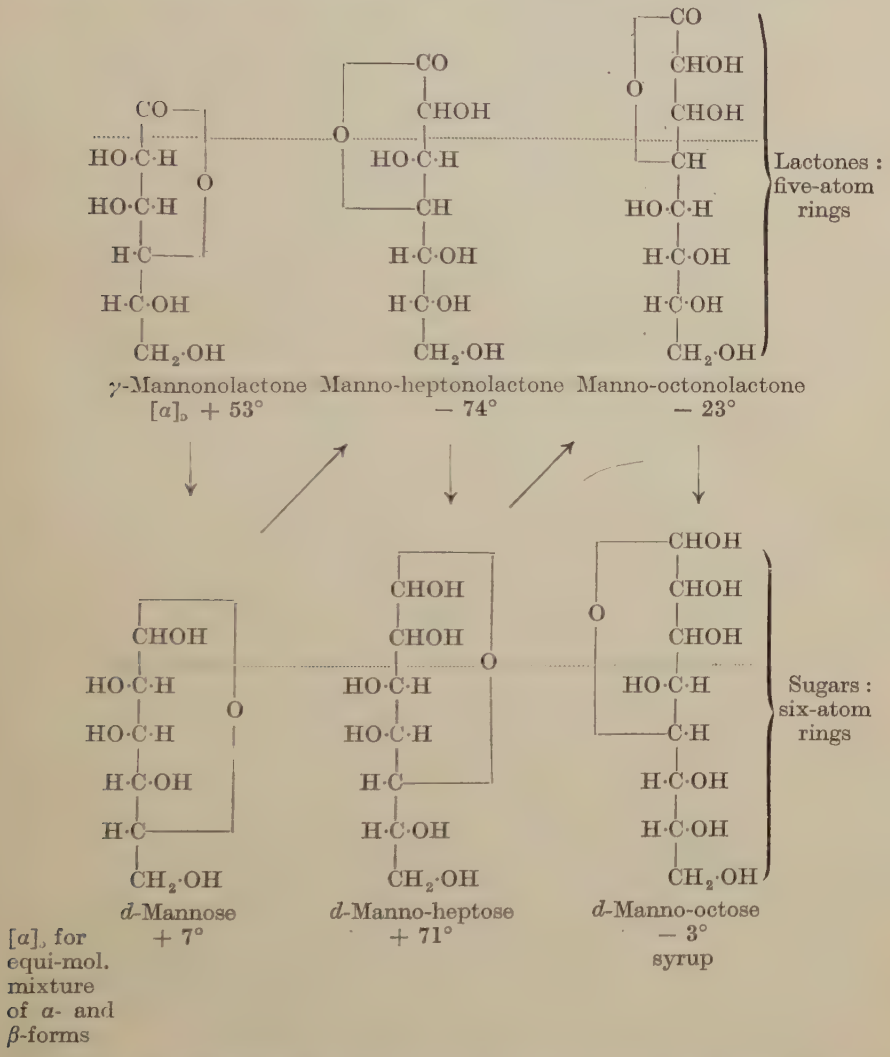
d-Mannose Series	Specific rotation
Mannohexonolactone	+ 53·81°
Mannose	+ 12·96°
Mannoheptonolactone	— 74·23°
Mannoheptose	+ 85·05°
Manno-octonolactone	— 43·58°
Manno-octose	— 3·3° (approx.)

"It is not quite understood at present what influences are active here. On the other hand, there can be no question that all the optical antipodes of the last four compounds can be synthesized from *l*-mannose." (Fischer included also manno-nonose in this table, but subsequently expressed doubt of the accuracy of the rotation quoted for it.)

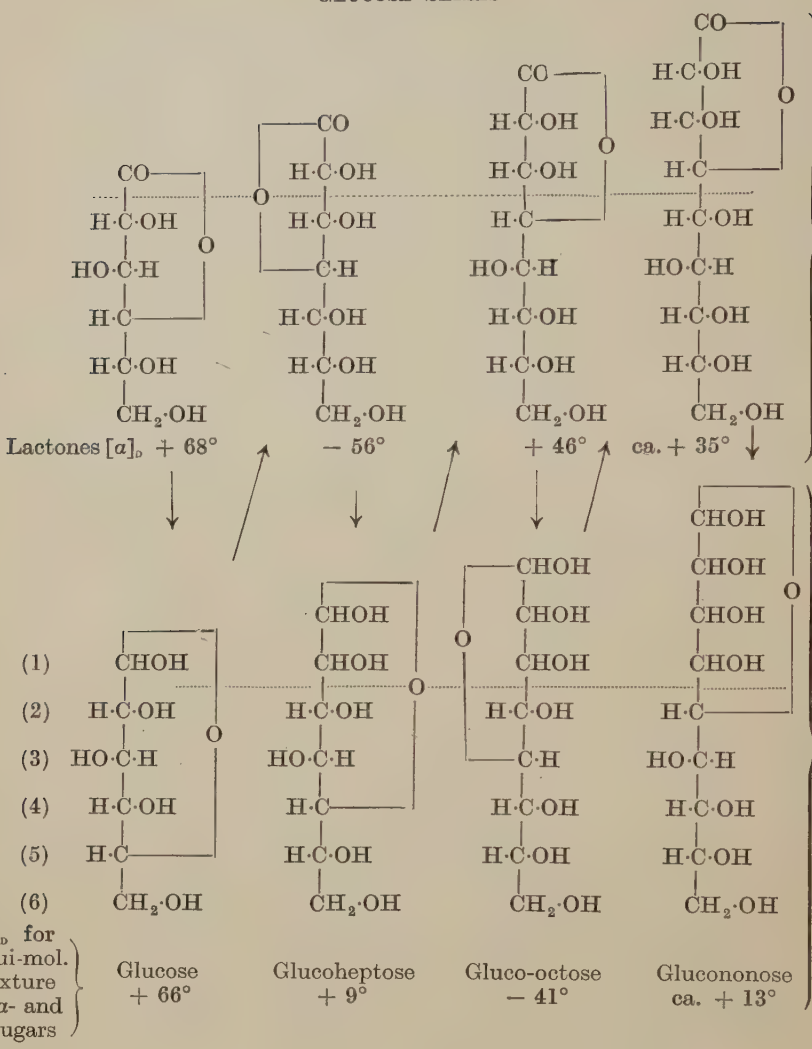
Whence arises this variation? An attempt to explain this marked divergence of rotatory tendency was made by Drew and Haworth [2] in a paper entitled *A Critical Study of Ring Structure in the Sugar Group*. They pointed out that if it be accepted that the lactones are five-atom ring structures and that these give rise on reduction to sugars having six-atom ring structures, the discrepancy which is disclosed in the preceding paragraph disappears, at least in all those cases for which direct comparisons could be made. Accepting this modification of structure for the sugar, one sees that the change over of sign remarked on by Fischer in the mannose series receives an

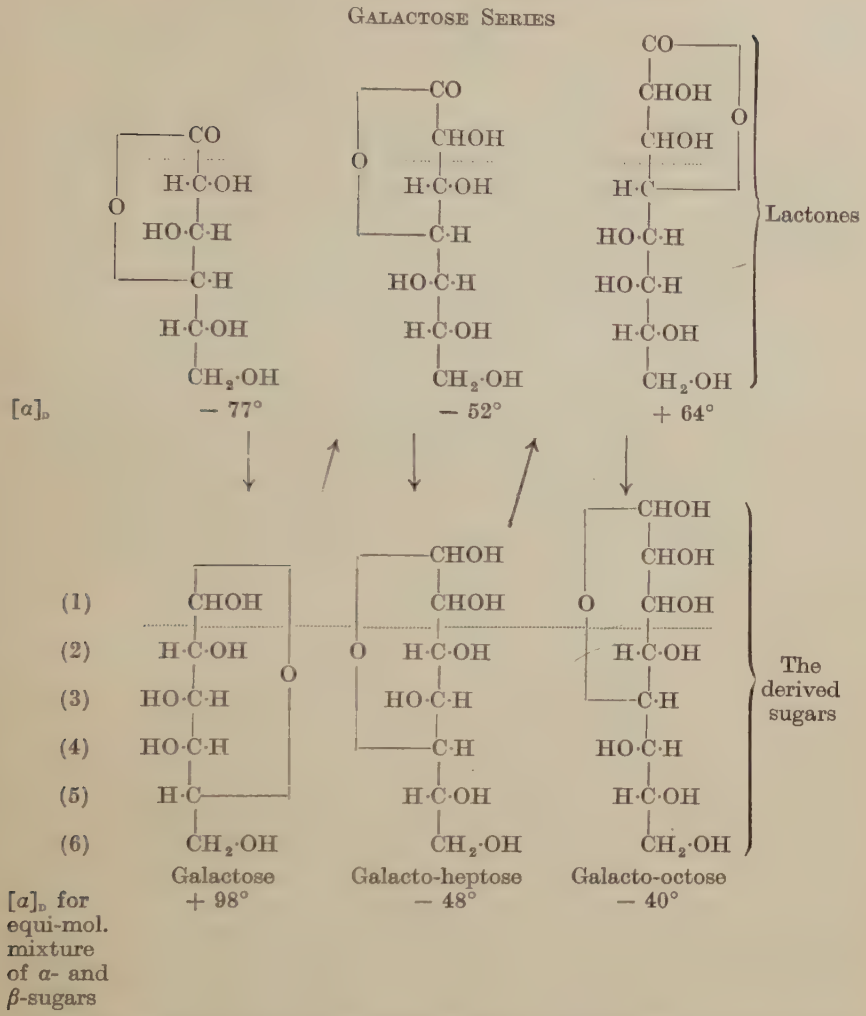
[1] *Untersuchungen über Kohlenhydrate und Fermente*, p. 582.
[2] *J.*, 1926, 2303.

explanation. The suggestion is indicated by the following projection formulæ, which show that when the oxide ring is to the right, the rotation is positive both for lactone and sugar ; and negative when the ring is to the left. The alternation in sign then follows the placing to the right or left of the hydroxyl which participates in ring formation.



A similar modification of the structure of sugars, from five- to six-atom rings, would also set right the optical anomalies which were apparent in the glucose and galactose series. These modified formulæ are given in the second and fourth lines below. Further examples in support of this working hypothesis are given by Drew and Haworth.


Glucose Series
Lactones
Lactones [α]D + 68°
− 56°
+ 46°
ca. + 35°
The derived sugars
(1)
(2)
(3)
(4)
(5)
(6)
[α]D for equi-mol. mixture of α- and β-sugars
Glucose + 66°
Glucoheptose + 9°
Gluco-octose − 41°
Gluconononose ca. + 13°



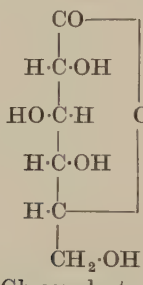
It would then appear that Hudson's lactone rule could not even remotely be applied in principle to the sugars themselves unless a different ring system from the five-atom ring structure be assumed for the sugars, and a six-atom ring structure meets the case.

It is not here implied that those cases for which data are at present lacking will necessarily conform to this simple rule, although no exception has yet been detected in those sugars for which numerous data are already available. This agreement must be regarded not necessarily as an argument for, but as a sequel to, the adoption of the six-atom ring structure for sugars. The arguments for the latter thesis will be developed in the subsequent pages. In pentoses the ring in the projection formulæ can be placed either to the right or the left

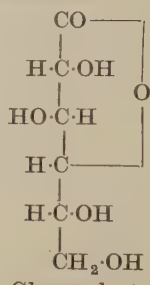
since the hydroxyl involved in ring formation is terminal in the chain and can freely rotate ; hence the normal pentoses can furnish no data either for or against this " rule."

These latter speculations were not advanced before evidence had been adduced from lactone study alone that the oxide rings in sugars could not be structurally identical with those assigned to γ-lactones. This evidence had indeed been neglected since 1914, when a paper appeared by J. U. Nef [1] describing the isolation of two additional lactones, other than γ-lactones, from the oxidation products of glucose and mannose. Nef had originally described the two new crystalline substances as β-gluconolactone and β-mannonolactone, but a study of their properties by Haworth and Nicholson [2] led to their recognition as δ-gluconolactone and δ-mannonolactone, each having a six-atom ring structure. The ease or difficulty with which they undergo hydration with consequent change of rotation furnished a ready means of distinguishing generally between a lactone of the five-atom ring type and a lactone of the six-atom ring type. These properties are summarized below.

LACTONES FROM GLUCOSE AND MANNOSE

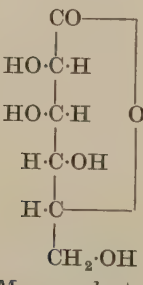


δ-Gluconolactone

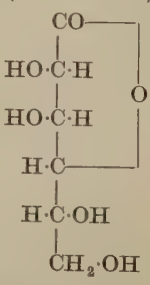


γ-Gluconolactone

	δ-Gluconolactone	γ-Gluconolactone
M.p.	152°	135°
$[\alpha]_D$ in water	$+ 63{\cdot}5° \longrightarrow 6{\cdot}2°$ (in $2\frac{1}{2}$ hours)	$+ 67{\cdot}8° \longrightarrow 58{\cdot}7°$ (in 24 hours)



δ-Mannonolactone



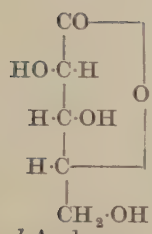
γ-Mannonolactone

	δ-Mannonolactone	γ-Mannonolactone
M.p.	156°	151°
$[\alpha]_D$ in water	$+ 114° \longrightarrow 27{\cdot}5°$ (in 22 hours)	$+ 51{\cdot}8°$ (no change in 24 hours)

[1] *loc. cit.* [2] *J.*, 1926, 1899.

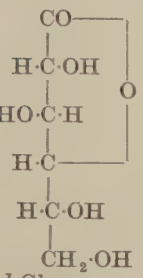
ADDENDUM

Glucose Series of Lactones



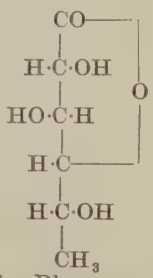
d-Arabono-

$[\alpha]_D$ + 73·7°



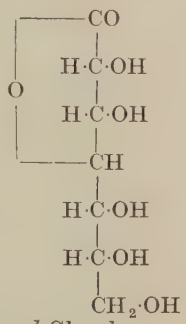
d-Glucono-

+ 68·2°



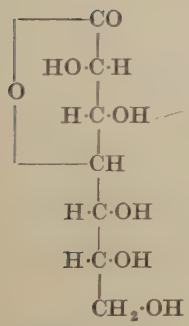
d-iso-Rhamnono-

+ 66·8°



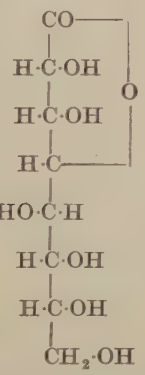
α-*d*-Glucohep-tono

− 56·0°



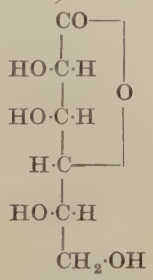
β-*d*-Glucoheptono-

$[\alpha]_D$ − 67·6°



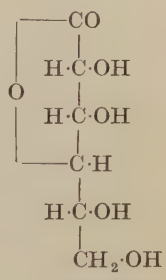
α-Gluco-octono-

+ 45·9°



l-Gulono-

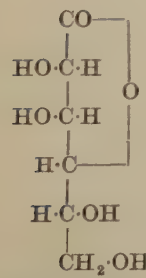
+ 55·1°



d-Gulono-

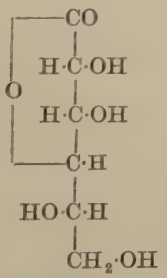
− 57·4°

Mannose Series of Lactones



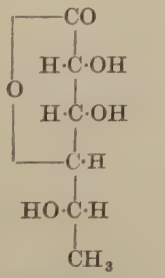
d-Mannono-

$[\alpha]_D$ + 53·8°



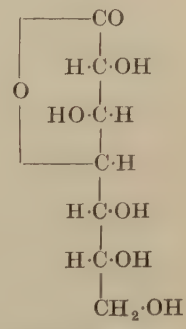
l-Mannono-

− 53·2°



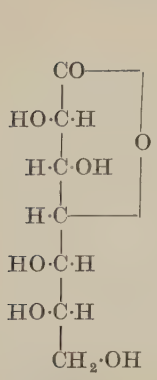
l-Rhamnono-

− 38·7°



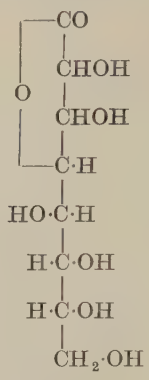
α-*d*-Mannoheptono-

− 74·2°



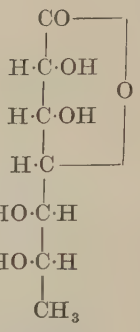
l-Manno-heptono-

$[\alpha]_D$ + 75·1°



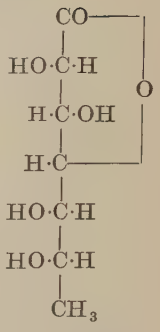
d-Manno-octono-

− 23·6°



β-*l*-Rhamno-hexono-

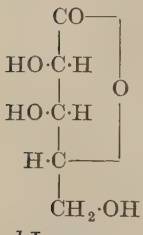
+ 43·3°



α-*l*-Rhamno-hexono-

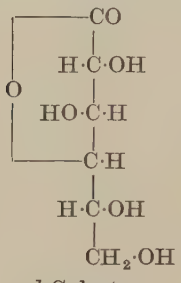
+ 86°

Galactose Series of Lactones



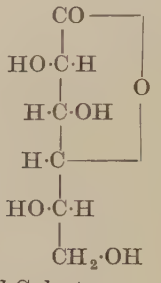
d-Lyxono-

$[\alpha]_D$ + 82·4°



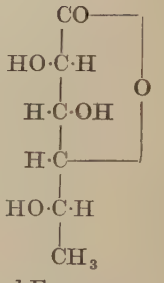
d-Galactono-

− 77·6°



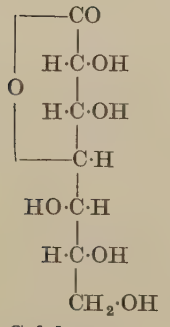
l-Galactono-

+ 72°



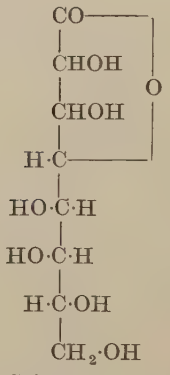
l-Fucono-

+ 78·3°



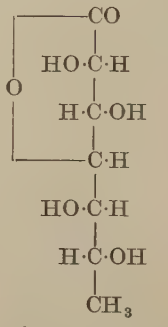
α-Galaheptono-

$[\alpha]_D$ − 52·3°



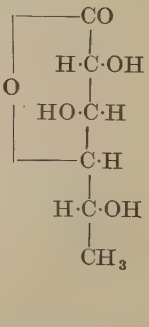
d-Gala-octono-

+ 64°



β-*d*-Fucohexono-

− 35°



d-Fuconolactone-

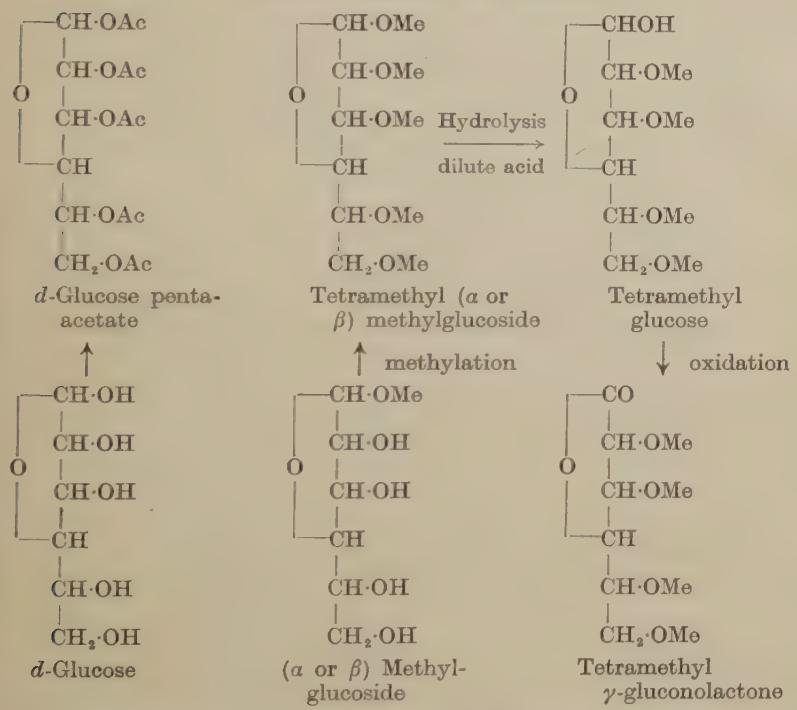
− 76·3°

CHAPTER III

INCONSISTENCIES IN THE OLDER FORMULÆ OF SUGARS AND THEIR METHYL DERIVATIVES

As an introduction to this chapter it is convenient to refer again to the constitutions ascribed to glucose and its derivatives on the basis of Fischer's formula for α- and β-methylglucosides, and to develop the study of the methylated lactones by exposing an inconsistency.

Older Formulæ of Fischer



As is well known from the work of Purdie [1] and his School, all the hydroxyl groups in glucosides are converted into methoxyl residues on methylation with methyl iodide and silver oxide. This result is accomplished with greater readiness by the use of methyl

[1] Purdie and Irvine, *J.*, 1903, **83**, 1021; Purdie and Bridgett, *J.*, 1903, **83**, 1037, *et seq.*

sulphate and aqueous alkali (Haworth [1]), and from the α- and β-methyl-glucosides the products obtained are the tetramethyl methylglucosides. Hydrolysis of the latter with dilute mineral acids leads to the elimination of the glucosidic methyl residue with the formation of crystalline tetramethyl glucose (normal).

The methylated sugar, the lactone and the glucose penta-acetate are represented here according to the older traditional formulæ which have been accepted without serious question and in the absence of rigid proof for several decades.

It has been emphasized in the preceding chapter that the evidence for the butylene oxide or five-atom ring formula of glucose, and its normal glucosides and derivatives, is founded on little more than an assumed analogy of their ring structure with that of the γ-lactones to which sugars most readily give rise on oxidation. This assumed analogy involves a very questionable principle. When a sugar is oxidized it changes to an open-chain monobasic acid. The ring system of the sugar is not preserved during this oxidation. But the open-chain acid is readily converted, even spontaneously on keeping, into the cyclic form of the lactone. In the above analogy it is supposed that open-chain polyhydroxy acids and aldoses exhibit the same tendency to undergo ring closure to identical cyclic forms. There may be some ground for this expectation as between aldoses and ketoses which are closely related compounds, but there is less immediate reason for anticipating the same consistency in type, character, or stability of ring forms as between, say, hexoses and their carboxylic acids.

This hypothesis has been the subject of an extended investigation involving the individual study of the lactones from methylated sugars. In the development of the problem it became clear that two structural varieties of lactones could be isolated: one type from those methylated sugars originating from normal methyl glucosides, and another type from the more recently discovered methylated sugars derived from the labile γ-glucosides (Chapter VI). Wide differences in properties characterized the two series of lactones. In selected cases a methylated sugar could give rise to lactones of both types. A significant example of this inter-relationship will now be described, and it will be apparent that a grave anomaly in the older constitutional formulations is revealed, but the examples will serve the purpose also of developing the characteristic behaviour and transformations of methylated sugars.

A partly methylated glucose was selected for this critical test.[2] This is referred to on pages 56 and 57, where its origin is outlined and the

[1] Haworth, *J.*, 1915, **107**, 8.

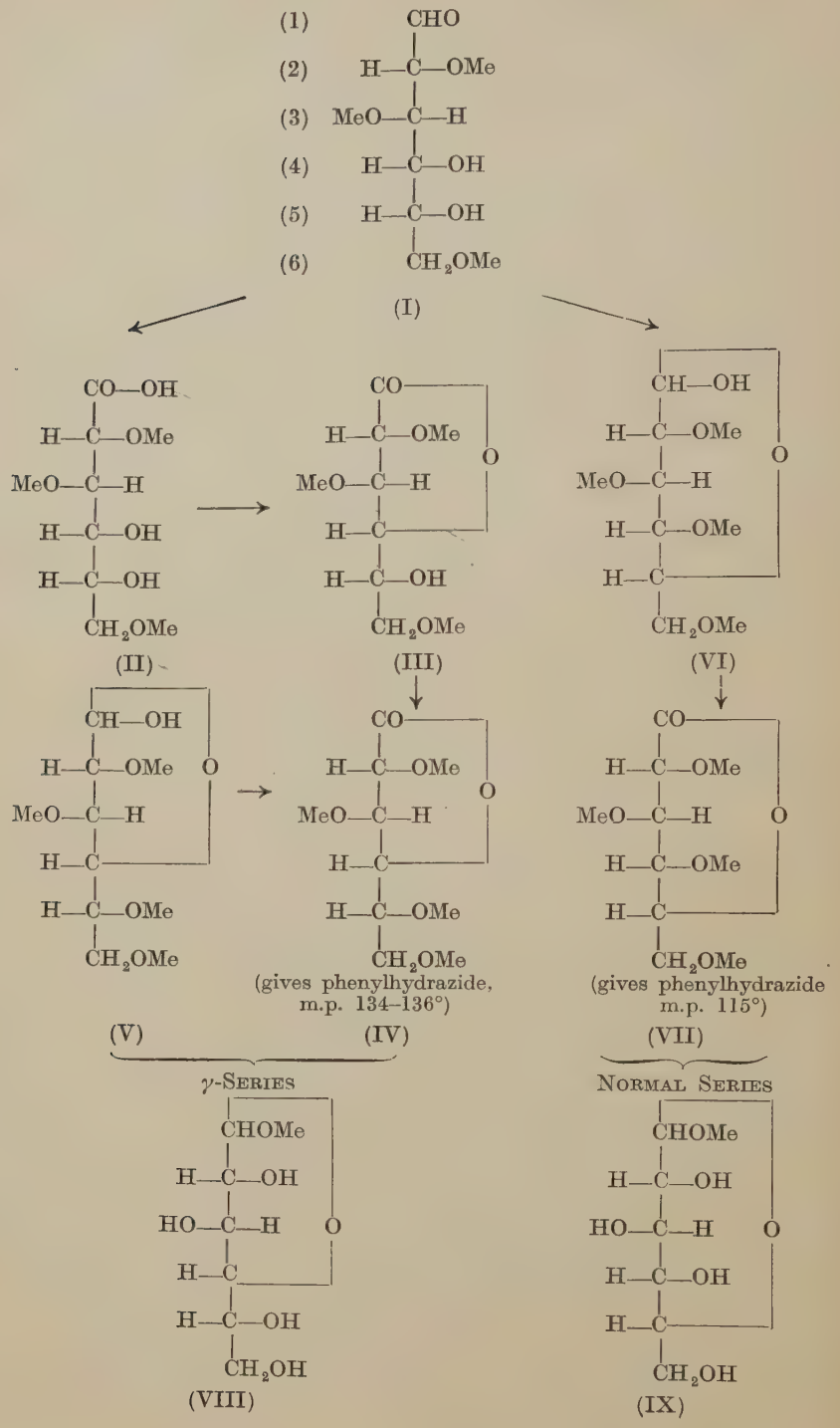
[2] Charlton, Haworth and Peat, *J.*, 1926, 89; Haworth, Hirst and Miller, *J.*, 1927, 2436.

positions of its substituent groups are definitely allocated. The example is 2 : 3 : 6-trimethyl glucose and, although this is known to possess an oxide-ring structure, it is preferable at this stage to represent the sugar by the open-chain formula (I) to avoid any anticipation of the issue.

Clearly the oxide ring can only occupy either of the alternative positions 1 : 4 or 1 : 5, since the remaining places in the chain are occupied by methoxy residues.

On oxidation with bromine water the sugar (I) is transformed into the 2 : 3 : 6-trimethyl gluconic acid (II). This was subjected to the experimental conditions which favour γ-lactone formation—rapid heating at 100°—when it was converted almost quantitatively into the lactone (III) which, on the principle of Hudson's lactone rule, is identified as 2 : 3 : 6-trimethyl γ-gluconolactone having the ring attached at positions 1 : 4. Methylation of the exposed hydroxyl group at position 5 gave the fully methylated 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone (IV), which is crystalline, and is further characterized by the ease with which it yields, by union with phenyl hydrazine, the crystalline phenyl hydrazide, m.p. 134–136°, of the corresponding acid. Here then we have a lactone of authentic structure, and this knowledge will be applied to advantage in the subsequent argument.

Now the original 2 : 3 : 6-trimethyl glucose (I) is readily converted by methylation into a tetramethyl methyl glucoside which yields on hydrolysis the normal crystalline tetramethyl glucose, identical with that obtainable from Fischer's α- or β-methyl glucosides under the appropriate procedure (page 18). On oxidation of this tetramethyl glucose a *liquid* tetramethyl gluconolactone was isolated which gave a crystalline phenyl hydrazide, m.p. 115°. Both lactone and phenyl hydrazide were widely different from the authentic specimen first described above and therefore the lactone could not have the structure of a γ-lactone although it is formulated as such (last formula on page 17), on the basis of the earlier constitutions assigned to α- and β-methyl glucosides by Fischer. None of these earlier structural formulæ is secure if the lactone now under discussion does not possess the butylene oxide 1 : 4-, or five-atom ring structure. Inspection of the formula of the original 2 : 3 : 6-trimethyl glucose reveals the only available alternative position for the oxide-ring, namely, 1 : 5, or an amylene oxide form, to which the six-atom ring is assigned both for the new tetramethyl lactone (VII) and for the crystalline tetramethyl glucose (VI) from which the lactone originated. It must follow that to Fischer's α- and β-methyl glucosides (IX) a like ring structure should be allocated.


(1) CHO
(2) H—C—OMe
(3) MeO—C—H
(4) H—C—OH
(5) H—C—OH
(6) CH2OMe
(I)
CO—OH
H—C—OMe
MeO—C—H
H—C—OH
H—C—OH
CH2OMe
(II)
CO
H—C—OMe
MeO—C—H
H—C
H—C—OH
CH2OMe
O
(III)
CH—OH
H—C—OMe
MeO—C—H
H—C—OMe
H—C
CH2OMe
O
(VI)
CH—OH
H—C—OMe
MeO—C—H
H—C
H—C—OMe
CH2OMe
O
(V)
CO
H—C—OMe
MeO—C—H
H—C
H—C—OMe
CH2OMe
O
(gives phenylhydrazide, m.p. 134–136°)
(IV)
CO
H—C—OMe
MeO—C—H
H—C
H—C—OMe
CH2OMe
O
(gives phenylhydrazide m.p. 115°)
(VII)
γ-Series
CHOMe
H—C—OH
HO—C—H
H—C
H—C—OH
CH2OH
O
(VIII)
Normal Series
CHOMe
H—C—OH
HO—C—H
H—C—OH
H—C
CH2OH
O
(IX)

The conclusion drawn from this preliminary work, combined with that outlined in the following chapter on the comparative rates of hydrolysis [1] of γ- and δ-lactones, was that the ring structure of normal glucosides and of glucose had been incorrectly assigned, and all carbohydrate formulæ were shown to have rested on a precarious basis. Some earlier preliminary studies [2] of xylose, arabinose and galactose also supported this conclusion.

It became abundantly clear that this was the case when it was demonstrated that the authentic γ-lactone (IV) could also be isolated by oxidation of the tetramethyl derivative (V) of the labile γ-glucose, and therefore that the 1 : 4-oxide or five-atom ring structure (VIII) was applicable to Fischer's new γ-methyl glucoside (page 42), and not to the normal structural forms of α- and β-methyl glucosides.

[1] Haworth, *Nature*, 1925, **116**, 430; Charlton, Haworth and Peat, *loc. cit.*

[2] Hirst and Purves, *J.*, 1923, 1352; Hirst and Robertson, *J.*, 1925, 358; Baker and Haworth, *ibid.*, p. 365. Compare Pryde, *J.*, 1923, 1808; and Haworth, Ruell and Westgarth, *J.*, 1924, 2468.

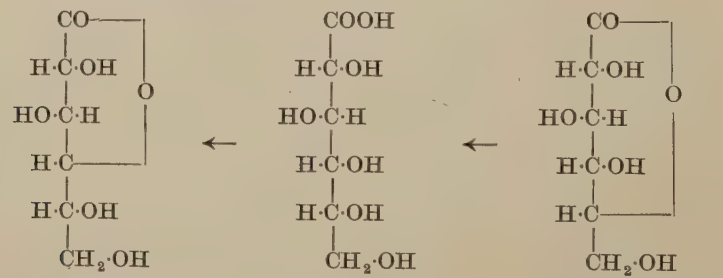
CHAPTER IV

METHYLATED LACTONES AND THEIR RELATION TO THE STRUCTURE OF SUGARS

The rate of hydration of a lactone to the open-chain acid can be measured by following the polarimetric changes (mutarotation) which occur in aqueous solution. An alternative method is to measure the increase of conductivity in water over comparative periods. Both these methods have been adopted in the study of a large series of lactones related to the sugars. The qualitative change effected in aqueous solution in the case of the two pairs of lactones derived from glucose and mannose is represented in fig. 1 on the next page.

In aqueous solution an equilibrium is reached between the monobasic acid and both the lactones. Knowing the specific rotation of the pure acid, it is possible to calculate with some accuracy the percentage amount of lactone present in solution at any given time. On page 24 the graphs for a number of lactones, which are methylated, are given.

Both the γ- and δ-gluconolactones are prepared from the same gluconic acid or its ester. Heating the acid at 100° for a short time favours formation of the γ-lactone. On the other hand, heating the ester, or the acid at a lower temperature for a prolonged period leads to the isolation of the δ-lactone.[1] The same holds for the two mannonolactones; and the δ-form can pass readily into the γ-form, since either hydroxyl group at position 4 or 5 is available for the linking with a carboxyl in ring closure.



γ-Gluconolactone — Gluconic acid — δ-Gluconolactone

For this reason a detailed study of lactones in which the hydroxyl

[1] Nef, *loc. cit.*; Hedenberg, *J. Amer. Chem. Soc.*, 1915, **37**, 345.

groups have been protected by substitution is rendered necessary. The methylated lactones are obtainable in two ways; (*a*) by direct methylation of an unsubstituted lactone, and (*b*) by the initial methylation of the sugar followed by oxidation to the lactone. Both methods of approach have been followed.

A series of methylated lactones derived from hexoses and pentoses

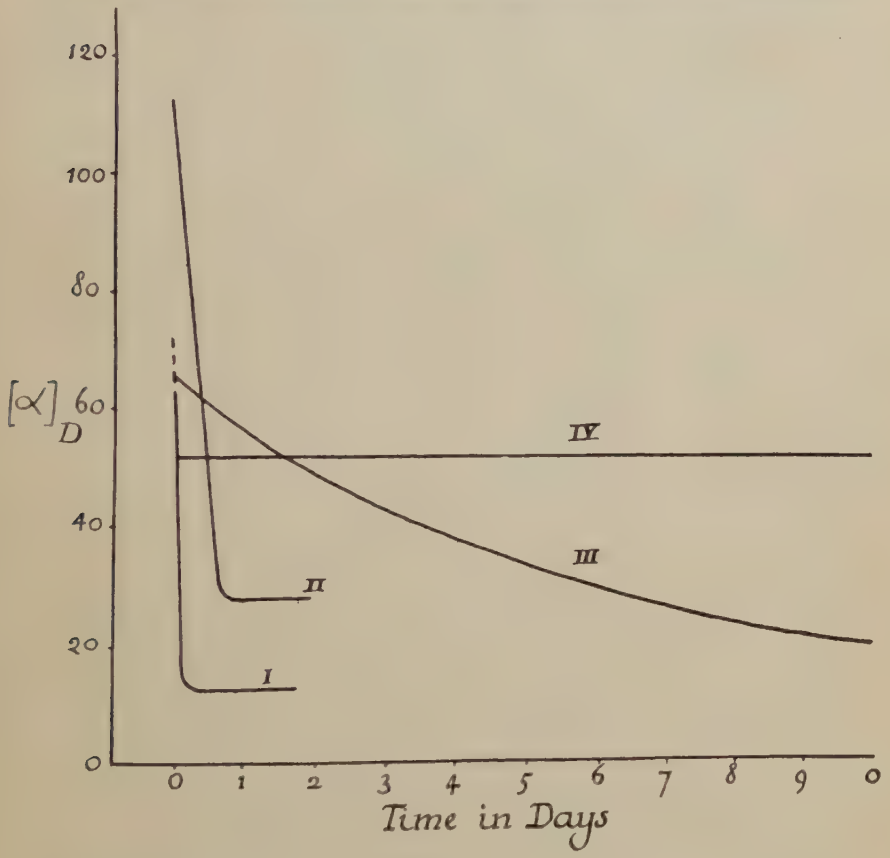


FIG. 1.

I δ-Gluconolactone. II δ-Mannonolactone.
III γ-Gluconolactone. IV γ-Mannonolactone.

displayed a physical behaviour which led to their characterization either as γ-lactones having a five-atom ring or as δ-lactones having a six-atom ring. The comparative rates of hydrolysis of a few of these lactones are indicated in the graphs below.[1] It is seen that the δ-lactones are much less stable than the γ-lactones, in water.

[1] Haworth, *Nature*, 1925, **116**, 430; Charlton, Haworth and Peat, *J.*, 1926, 89; Haworth and Westgarth, *ibid.*, p. 880; Haworth and Nicholson,

Fortunately it was possible to base these comparisons on definite oxidation experiments conducted on a representative γ-lactone and a representative δ-lactone. Thus, the crystalline trimethyl γ-arabonolactone was characterized by its oxidation to 2 : 3-dimethoxy-4-hydroxy glutaric acid,[1] and the formation of this product was accompanied by loss of a methoxy residue at the fifth position in the carbon chain. Since this lactone was initially derived from the labile form and not

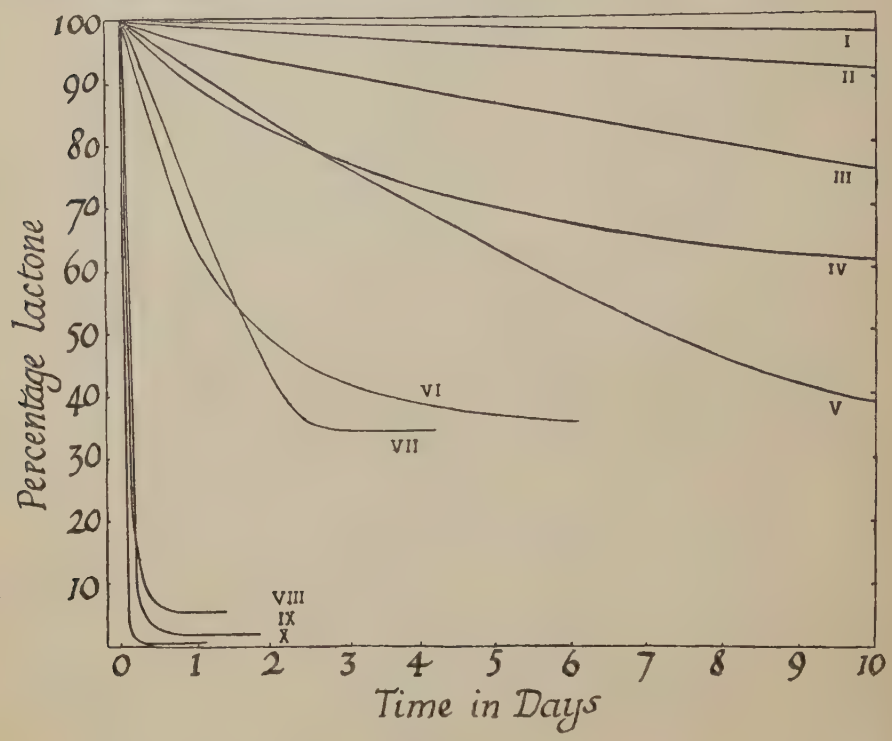


Fig. 2.

I	Tetramethyl γ-mannonolactone	VI	Tetramethyl δ-mannonolactone
II	Tetramethyl γ-galactonolactone	VII	Trimethyl δ-xylonolactone
III	Trimethyl γ-xylonolactone	VIII	Tetramethyl δ-gluconolactone
IV	Trimethyl γ-arabonolactone	IX	Tetramethyl δ-galactonolactone
V	Tetramethyl γ-gluconolactone	X	Trimethyl δ-arabonolactone

the normal α- or β-form of methylarabinoside, it was evident that the latter could not be formulated on the basis of a γ-lactone.

The trimethyl δ-arabonolactone was investigated in the same

ibid., p. 1899 ; Levene and Simms, *J. Biol. Chem.*, 1926, **68**, 737 ; Drew, Goodyear and Haworth, *J.*, 1927, 1237 ; Haworth and Porter, *J.*, 1928, 611 ; Haworth, *Helv. Chim. Acta*, 1928, **11**, 534.

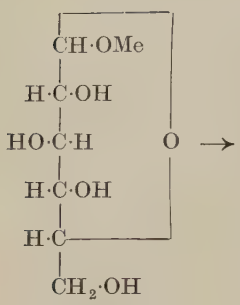
[1] Baker and Haworth, *J.*, 1925, 365 ; Haworth and Nicholson, *loc. cit.*

way and this gave [1] on oxidation with nitric acid the optically active arabo-trimethoxy glutaric acid (see page 29). The latter product had also been obtained directly [2] by the oxidation of normal trimethyl arabinose, which is itself prepared from α- and β-methyl arabinosides. It was therefore possible to compare the hydration curves of methylated γ-lactones with unsubstituted γ-lactones whose constitution rested until this stage on the truth of Hudson's lactone rule. The tetramethyl δ-glucono-, galactono-, and the unsubstituted δ-mannonolactone showed hydration curves comparable in all respects with that determined for trimethyl δ-arabonolactone, and it is seen that from the oxidation results just described the latter can only be represented structurally by the six-atom ring.

Convincing as these comparisons seemed to be, it was necessary to seek further confirmation of the important conclusions by the adoption of direct methods of degradation through oxidation processes; and also secondly to strengthen the reasoning by bringing to bear processes of synthesis in so far as these could be achieved. All these methods of approach to a solution of the constitutional problem have been followed. The results of the oxidation of the various lactones provide a complete verification of the inferences drawn from the physical properties of the lactones. The degradation method has been applied to the lactones from glucose,[3] xylose,[4] galactose,[5] arabinose,[6] mannose,[7] fructose,[8] rhamnose [9] and lyxose.[10] But space allows us to outline here the results in only one or two representative cases.

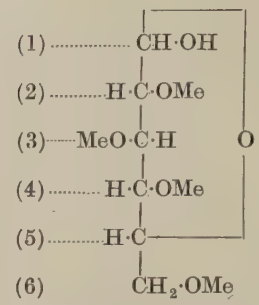
By employing Fischer's α- and β-methylglucosides (I) as the starting point of the series, and proceeding to the methylation of these to the tetramethyl methylglucosides, it is possible as already shown (page 18) to isolate the representative methylated sugar (II), namely crystalline tetramethyl glucose (normal). This is converted by mild oxidation to the corresponding tetramethyl lactone (IV) and, by a further stage of oxidation with concentrated nitric acid, to xylotrimethoxyglutaric acid (V). The same final product can be achieved by oxidizing the methylated sugar initially with nitric acid.[11]

[1] Haworth and Jones, *J.*, 1927, 2349. [2] Hirst and Robertson, *J.*, 1925, 358.
[3] Haworth, Hirst and Miller, *loc. cit.*
[4] Haworth and Jones, *loc. cit.*; Haworth and Porter, *loc. cit.*
[5] Haworth, Hirst and Jones, *J.*, 1927, 2428.
[6] Haworth and Jones, *loc. cit.*; Haworth and Nicholson, *loc. cit.*; Haworth, Hirst and Learner, *J.*, 1927, 2432.
[7] Goodyear and Haworth, *J.*, 1927, 3136.
[8] Haworth, Hirst and Learner, *J.*, 1927, 1040; Haworth, Hirst and Nicholson, *J.*, 1927, 1513; Avery, Haworth and Hirst, *ibid.*, p. 2308.
[9] Avery, Haworth and Hirst (*in the press*).
[10] Haworth, Hirst and Smith (*in the press*); Hirst and Smith (*in the press*).
[11] Hirst, *J.*, 1926, 350.



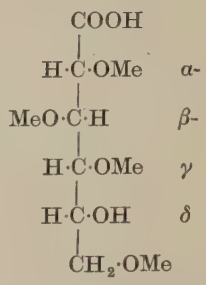
α- and β-Methyl glucoside

(I)



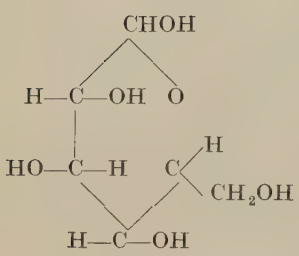
Tetramethyl Glucose 2 : 3 : 4 : 6 (crystalline)

(II)



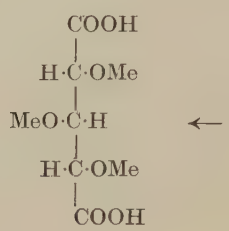
2 : 3 : 4 : 6-Tetramethyl gluconic acid

(III)



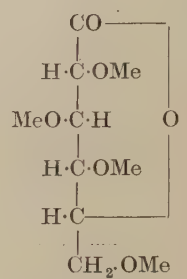
Glucose

(VI)



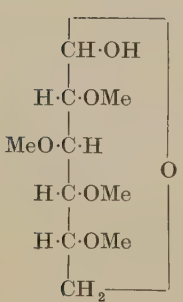
Xylo-trimethoxy-glutaric acid

(V)

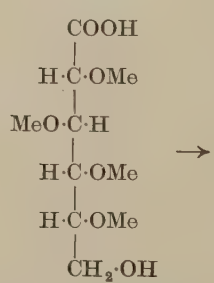


Tetramethyl δ-Gluconolactone

(IV)

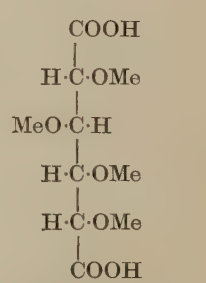


(VII)



2 : 3 : 4 : 5-Tetramethyl gluconic acid

(VIII)



Tetramethyl Saccharic acid

(IX)

This result of degradation shows definitely that the oxide-ring cannot engage the first and fourth carbon atoms and that it must be attached either at the fifth or sixth carbon atom of the hexose chain. Fortunately the latter alternative (VII) was easily excluded by a large number of observations; for example, methylglucoside undergoes oxidation at the sixth position to the methylglucoside of glycuronic acid.[1] Secondly, 6-triphenylmethyl glucose has been acetylated and from this product a triphenylmethyl residue is easily displaced, yielding a triacetyl glucose which passes to *iso*rhamnose on reduction of the corresponding halogen derivative.[2] The same triphenylmethyl glucose changes to trimethyltriphenylmethyl glucose on methylation, and elimination of the triphenylmethyl residue leads to 2 : 3 : 4-trimethyl glucose,[3] which is oxidized to give xylo-trimethoxyglutaric acid, or alternatively, may be methylated to give crystalline tetramethyl glucose (normal).

Perhaps the most convincing proof that the oxide-ring does not engage position 6 as shown in formula (VII), but is attached at position 5 of the chain, is the isolation from quite independent sources [4] of a 2 : 3 : 4 : 5-tetramethyl gluconic acid (VIII) which cannot under any experimental conditions be made to pass into tetramethyl δ-gluconolactone. Oxidation leads to its conversion into 2 : 3 : 4 : 5-tetramethyl saccharic acid (IX) and not to trimethoxyglutaric acid. Crystalline derivatives of all these latter products are available for purposes of direct comparison.

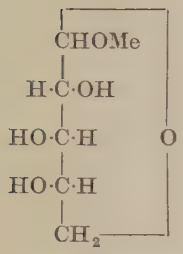
We have, then, eliminated from consideration the possibility of a seven-atom ring and of a three-, four- or five-atom ring. The only remaining position for the attachment of the oxide-ring in tetramethyl glucose is therefore at the first and fifth carbon atoms of the chain, giving a six-membered ring compound as illustrated in the preceding formula (II). It follows that α- and β-methylglucosides have the same ring structure which is shown in formula (I). These glucose derivatives have as their parent sugar the six-atom ring form of glucose which is indicated by formula (VI).

The constitutional proof is developed with even greater clearness in the cases of the pentose derivatives. In a pentose, such as arabinose, no possibility of a seven-atom ring can arise inasmuch as the ring-forming oxygen can only join the two ends of a five-carbon chain or connect intermediate carbon atoms of the chain. The heterocyclic form of a pentose sugar cannot therefore exceed six component atoms. Such a stabilized ring form is found in the α- and β-methylarabinosides

[1] Smolenski, *Rocz. Chem.*, 1923, **3**, 153.
[2] Helferich, Klein and Schäfer, *Annalen*, 1926, **447**, 19.
[3] Haworth, Hirst and Miller, *loc. cit.*; also Haworth and Learner, unpublished results.
[4] Haworth, Loach and Long, *J.*, 1927, 3146.

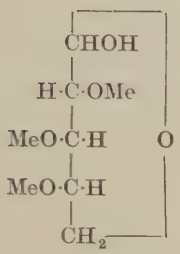
(X) which were originally prepared from *l*-arabinose by E. Fischer. Digestion of the pentose with methyl alcohol containing 0·5 per cent. hydrogen chloride leads to the isolation of both stereochemical varieties which are crystalline. By methylation with methyl sulphate each gives rise to a trimethyl methylarabinoside, and hydrolysis of either methylated product yields one and the same form of *l*-trimethyl arabinose (XI). This is a characteristic and normal sugar. It undergoes oxidation with bromine water to the corresponding acid (XII) which passes quantitatively into the crystalline *l*-trimethyl δ-arabonolactone [1] (XIII). The properties of the latter are of special interest in another connexion (see page 79), and it should be particularly emphasized that the rate of hydration (page 24) of this lactone is almost identical with that of the tetramethyl δ-gluconolactone (IV) derived from normal crystalline *d*-tetramethyl glucose (II). On further oxidation with nitric acid this lactone passes into *l*-arabo-trimethoxy glutaric acid (XIV) which is also obtained by direct oxidation of the sugar.[2] The parent sugar form of all this series of substances is therefore the six-atom ring type of arabinose (XV) which is more accurately expressed by the hexagon formula (XVI). This is evidently the naturally occurring variety of arabinose.

[1] Haworth and Jones, *loc. cit.* [2] Hirst and Robertson, *loc. cit.*



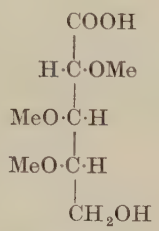
Normal *l*-methyl-arabinosides

(X)



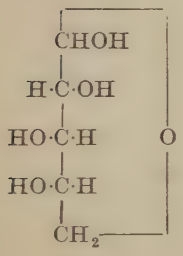
l-Trimethyl arabinose

(XI)

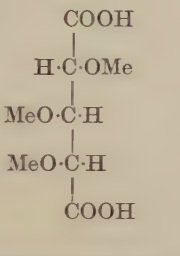


Trimethyl arabonic acid

(XII)

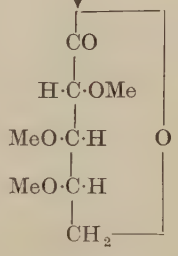


(XV)



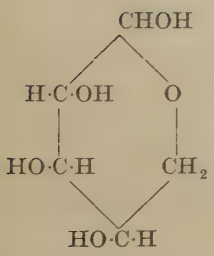
l-Arabo-trimethoxy-glutaric acid

(XIV)



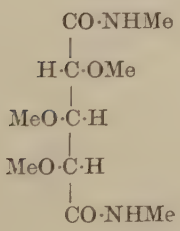
l-Trimethyl δ-arabonolactone

(XIII)



l-Arabinose

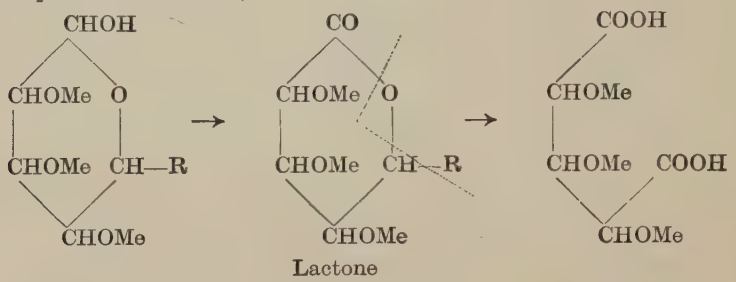
(XVI)



(crystalline, m.p. 172°)

By following a like procedure in the cases of trimethyl xylose, trimethyl lyxose, trimethyl rhamnose, tetramethyl galactose and tetramethyl mannose, the same general conclusion as a principle of ring structure has been established. All these methylated sugars have the general formula (XVII) and are prepared in the first instance from the normal methylpentosides or methylhexosides. Each of these on oxidation gives rise to a methylated δ-lactone which, by further oxidation with nitric acid, is changed into a trimethoxyglutaric acid (XVIII).

(R = H, in a pentose ;
(R = CH_2OMe in a hexose.)



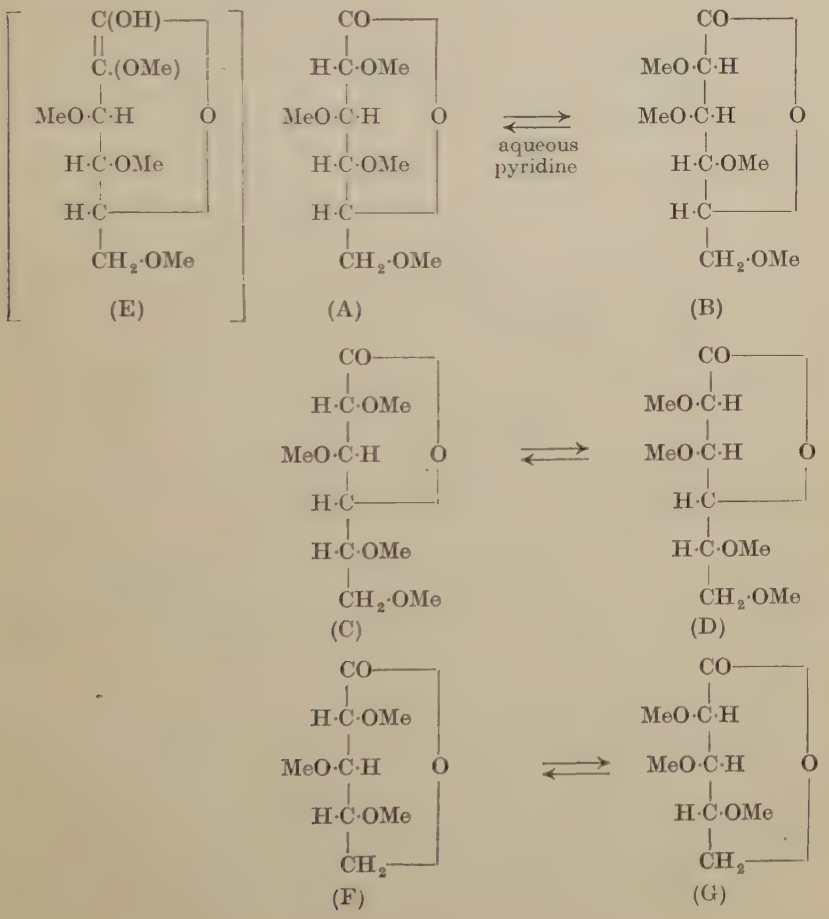
(XVII) (XVIII)

The orientation of the methoxyl groups differs in the latter product according as the distribution of the hydroxyls in the parent sugar is from left to right. Thus the same crystalline methylamide of trimethoxyglutaric acid as that isolated from methylated glucose was also obtained from methylated xylose, whilst that from methylated arabinose was identical with that from methylated galactose. On the other hand, methylated *d*-mannose gave, through its δ-lactone, a trimethoxyglutaric acid which was the optical enantiomorph [1] of that obtained from methylated galactose. Importance is obviously attached to the precise recognition of the appropriate active forms which may be expected from the configuration of the parent sugar.

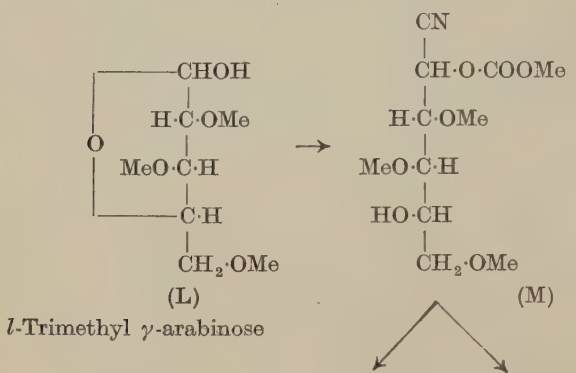
Supplementary evidence of a confirmatory character served also to strengthen the views of constitution which have here been developed. The complementary facts which are now to be outlined are of service in that they duplicate the various proofs which have been adduced of the six-atom ring structure of individual sugars. It has been emphasized that the isolation of a trimethoxyglutaric acid of the appropriate stereochemical variety is an essential part of the argument in assigning constitutions to the lactone and sugar. The following experiments [2] show that the stereochemical identity of

[1] Goodyear and Haworth, *J.*, 1927, 3136.
[2] Haworth and Long (*in the press*).

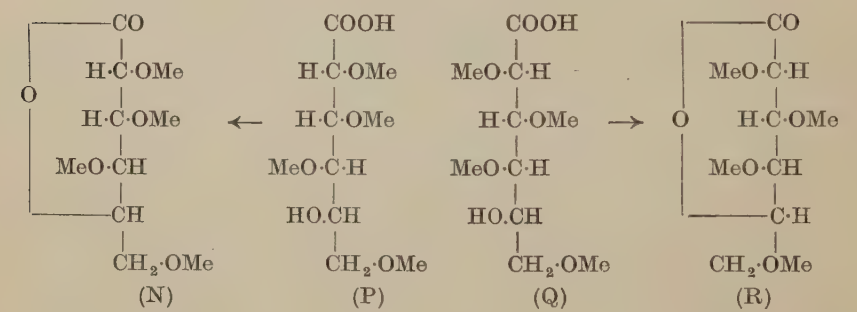
the lactones can be established by their interconversion from one to another by the process of epimerization. This process may be considered as proceeding either through the enolic form of the lactone (E) or through the corresponding acid. In each case the effect is to transpose a methoxyl group from its relative spatial position with respect to the hydrogen atom attached to the α-carbon atom. Thus, tetramethyl δ-gluconolactone (A) undergoes epimerization to tetramethyl δ-mannonolactone (B), the equilibrium lying on the side of the formation of a greater quantity of the latter product. Similarly, the tetramethyl γ-mannonolactone (D) is changed by epimerization to the γ-gluconolactone (C). In the same way also trimethyl δ-xylonolactone (F) has been converted into trimethyl δ-lyxonolactone (G). These results serve to correlate lactones of like structure but differing stereochemical identity, thus affording confirmatory evidence of the same type of ring in each pair.



It seemed prudent not only to place reliance on the physical characteristics of lactones and on their products of degradative oxidation, but also to endeavour to gain an insight into carbohydrate structure by methods of synthesis. An alternative proof would be to achieve the synthesis of a tetramethyl glucose or tetramethyl mannose from, for example, *d*-tartaric acid. This has not yet been accomplished, but certain essential stages have been attained, and the following experiments illustrate the synthesis of a six-atom ring derivative of a hexose from a pentose derivative having the five-atom ring structure.



l-Trimethyl γ-arabinose



l-Tetramethyl δ-mannolactone

Phenylhydrazide showed $[\alpha]_D$ + 22° and m.p. 183–184° as compared with that from *d*-series $[\alpha]_D$ − 22°, m.p. 183–184°.

l-Tetramethyl δ-gluconolactone

Phenylhydrazide showed $[\alpha]_D$ − 50°, m.p. 115° as compared with that from *d*-series, $[\alpha]_D$ + 50·8°, m.p. 115°.

Baker and Haworth showed that when *l*-arabinose is condensed with methyl alcohol at ordinary laboratory temperature in presence of 1 per cent. hydrogen chloride, the major product consists of a γ-methylarabinoside which is different from the known α- and β-forms of methylarabinoside. The new variety belongs to the category of the γ-sugar derivatives and is therefore related to Fischer's γ-

methylglucoside (see page 43). Methylation of the γ-methylarabinoside gives rise to a trimethyl γ-arabinose, and the proof of the constitution (L) given below for this substance is outlined on page 44. It will be seen that this is represented as a five-atom ring structure, contrasting with that of the six-atom ring structure of normal sugars. The relationship of this type of so-called γ-sugar derivative to a normal sugar has been tested by ascent [1] of the series from trimethyl γ-arabinose (L) to tetramethyl *l*-gluconolactone (R) and to tetramethyl *l*-mannonolactone (N). Condensation of the trimethyl γ-arabinose with ethyl chloroformate and potassium cyanide gave a product of formula (M) which was converted into a mixture of trimethyl gluconic acid and trimethyl mannonic acid. The separation of these two substances was followed by transformation into the tetramethyl acids (Q). (P), and finally to the lactones. The identity of the lactones was established by conversion to the crystalline phenylhydrazides. They were found to be structurally identical with those of *d*-tetramethyl δ-gluconolactone and *d*-tetramethyl δ-mannonolactone (see page 26), having the same specific rotation and melting point and differing only in the sign of optical rotation.

[1] Haworth and Peat (*in the press*).

CHAPTER V

NORMAL FRUCTOSE. THE GENERALIZATION THAT NORMAL SUGARS ARE RELATED TO PYRAN (THE PYRANOSE SERIES)

To conclude the present line of discussion, reference need now only be made in the hexose series to the representative ketose, namely ordinary fructose. This crystalline sugar yields two methylfructosides, which are α- and β-forms, and both are crystalline.[1] The β-methylfructoside (XX) is readily converted into a crystalline [2] tetramethyl fructose (XXI). It will be obvious that oxidation of fructose could not be expected to lead to a hexono-lactone similar in type to those derivable from the aldoses, since the reducing group is not in a terminal position. Indeed, the action of bromine water on tetramethyl fructose is inappreciable, and even with the unmethylated sugar little change occurs, except after a prolonged period of contact of many weeks. Crystalline tetramethyl fructose has been found to be a more stable sugar in this instance than the corresponding glucose derivative. But on digestion with nitric acid, dilute or concentrated, tetramethyl fructose (XXI) undergoes a very characteristic change. This oxidation is effective at the first carbon position, which may indeed be regarded as a side-chain of a ring. Under this treatment [3] the terminal $-CH_2OMe$ group is transformed into a carboxyl group and the existence of crystalline methyl and ethyl esters of this monobasic acid (XXII) renders its identification a simple one. As a by-product of this oxidation process there is also isolated arabo-trimethoxyglutaric acid, recognizable through its crystalline methylamide. From the constitutional point of view an even more instructive degradation can be effected by oxidation of the monobasic acid (trimethyl fructuronic acid) with acidified permanganate solution. Under these conditions *d*-2 : 3 : 4-trimethyl δ-arabonolactone (crystalline) is formed,[4] and this (XXIII) is the optical enantiomorph of the product derived by the action of bromine water on *l*-trimethyl arabinose (XIII). That this is indeed the case was finally proved

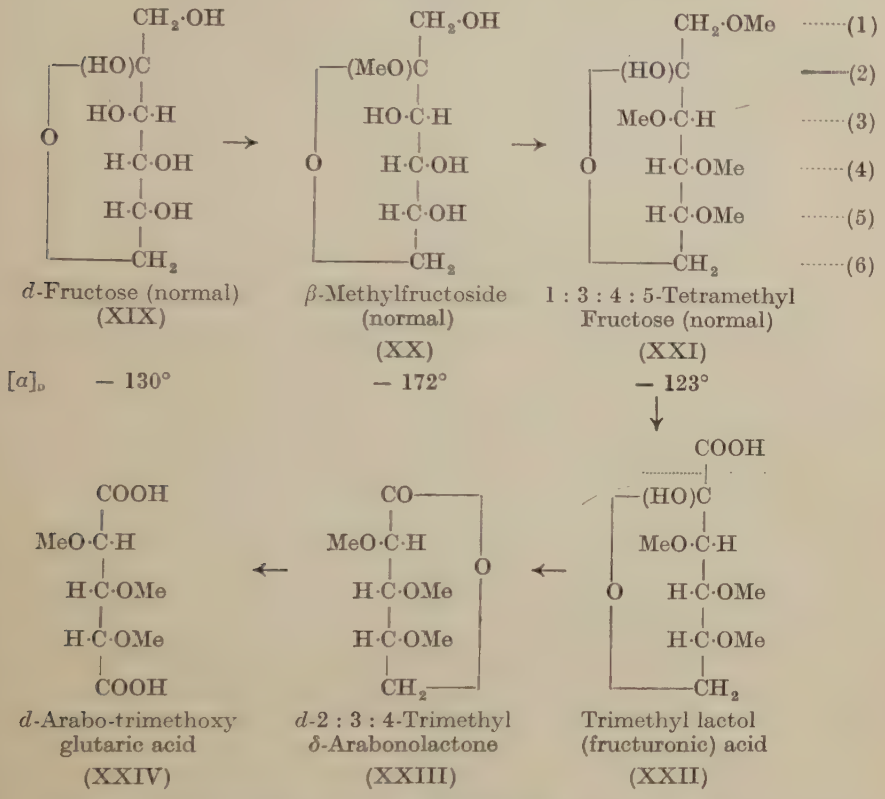
[1] Hudson, *J. Amer. Chem Soc.*, 1916, **38**, 1216. Schlubach and Schröter, *Ber.*, 1928, **60**, 1216.

[2] Purdie and Paul, *J.*, 1907, **91**, 289; Miss E. S. Steele, *J.*, 1918, 257.

[3] Haworth and Hirst, *J.*, 1926, 1858; Haworth, Hirst and Learner, *J.*, 1927, 1040.

[4] Haworth and Nicholson (*in the press*).

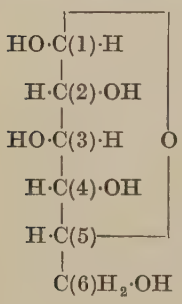
on further oxidation of the lactone to *d*-arabo-trimethoxyglutaric acid (XXIV), which has a rotation equal in magnitude but opposite in sign to that of the *l*-variety (XIV), obtainable by oxidation of *l*-trimethyl arabinose or its lactone.



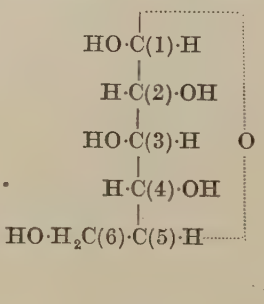
The significant fact emerges that normal fructose and its crystalline methylfructoside must be structurally represented by a six-atom ring. It follows therefore that all the representative hexoses, namely, glucose, fructose, galactose, mannose, as also the pentoses arabinose, xylose and lyxose, and the methyl pentose, rhamnose, exist in the form of their normal methylhexosides or pentosides as six-atom ring structures. There can be little doubt that the free sugars also have a like structure. In the case of the aldoses which have been mentioned, definite evidence has been forthcoming that where glucose or galactose exists in combination in a disaccharide such as maltose, cellobiose, gentiobiose, lactose and melibiose, the hexose structures are invariably the same as those which have already been discussed; that is, the forms of these sugars which occur naturally in disaccharides are those of a six-atom ring structure indicated below. The

example given in the following formulæ is that of the characteristic β-glucose. The first of the three formulæ is written in the conventional manner. It is important to remark, however, that this formula does not indicate in a true sense the spatial distribution of groups at the fifth carbon atom. If a model of the conventional aldehyde form be constructed and, with this model, ring closure is effected between the aldehyde group and the hydroxyl at C_5, it will be found that in the act of effecting ring closure the terminal —CH_2OH group swings out to the left and the hydrogen atom on this carbon will now occupy a reversed position from that indicated in the projection formula (I). The ring is now indicated in formula (II) by dotted lines for the reason that the oxygen must align itself in the same plane as the five carbon atoms of the ring. Similarly the lactone ring closure will effect the same distribution of groups at the fifth carbon atom. It may be that the enhancement of rotation which accompanies ring closure of a hexonic acid is attributable to the spatial change which is here involved.

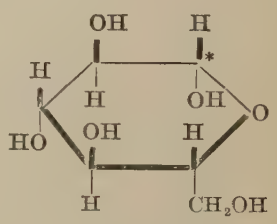
Probably a clearer mode of expression for the six-atom ring with its addenda is that given in the third formula below, where the ring is placed on its side and is viewed in perspective. Here it may be interesting to remark that the correct formulation of β-glucose requires that the hydrogen atoms associated with the five carbon atoms are alternately above and below the plane of the ring. This gives a completely *trans* distribution for the groups in the sugar which, as condensed residues in cellulose, occurs most widely in nature.



(I)



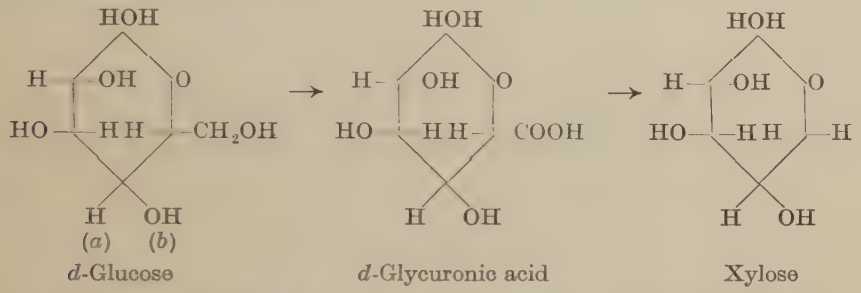
(II)



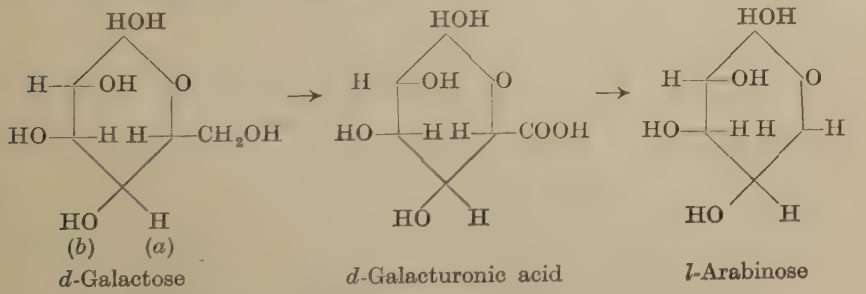
β-Glucose. (III)

It follows that the most appropriate symbol for the formulation of a normal sugar, and one that portrays most clearly the atom-model constructed on ordinary principles of valency distribution, is that which represents the six-atom ring as a hexagon. Such a formula has the merit also of illustrating with greater clearness many of the reactions which sugars undergo. A photograph of such a model is given (*Frontispiece*) and the sides of the hexagon may be regarded as imaginary lines joining the centres of the five carbon atoms and

one oxygen atom. The sixth carbon atom is seen to present itself in the formula below as a side-chain or primary alcohol group. This alcohol group undergoes, under appropriate conditions, oxidation to a carboxyl group, leading to the formation of glycuronic acid. Thus, if camphor or menthol is introduced into the food of some animals they are able to eliminate it in the form of campho- or menthol-glycuronic acid, which on hydrolysis give crystalline glycuronic acid. In contact with certain bacteria glycuronic acid loses carbon dioxide and passes into xylose.

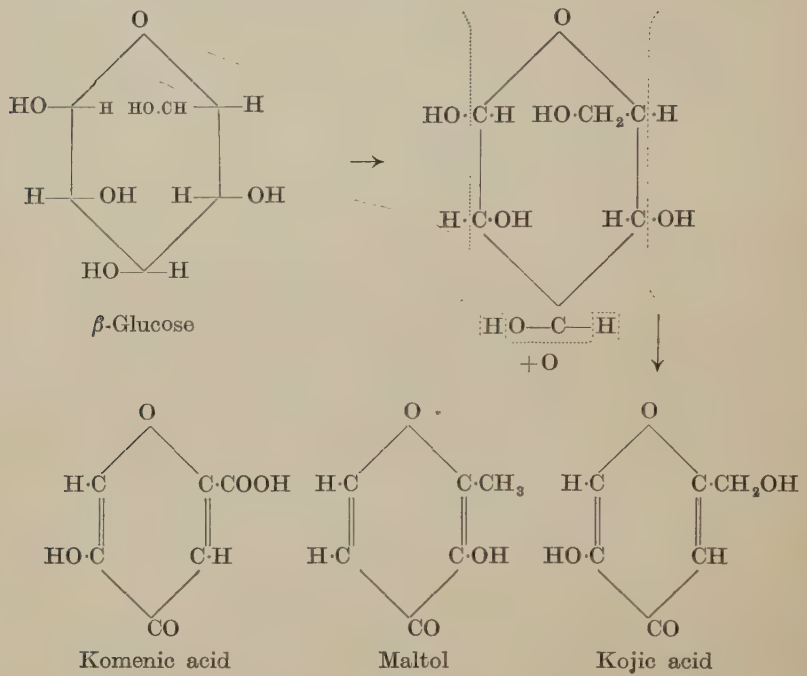


On a similar plan it is easy to explain the occurrence together in plant products of *d*-galactose, *d*-galacturonic acid and *l*-arabinose. Inasmuch as glucose gives rise, on descent of the series (page 6), to *d*-arabinose, it has been difficult to account for the occurrence of *l*-arabinose so freely in nature, and for the rarity of *d*-arabinose. The reason may be sought in the capacity of galactose to undergo ready transformation in the plant to galacturonic acid, and then, by loss of carbon dioxide, to *l*-arabinose.[1] This is a change which recalls that of benzyl alcohol to benzoic acid and the formation from the latter of benzene. Probably glucose is the earliest of the simple sugars to be formed in nature, and is to be regarded as the precursor of other sugars. By some simple mechanism in the plant or animal the inversion of the hydrogen and hydroxyl at (*a*) (*b*) takes place (see glucose formula above) in such a way that the hydroxyl in galactose appears on the left of carbon atom 4 instead of on the right as in glucose.



[1] Haworth, *J. Soc. Chem. Ind.*, 1927, **46**, 295 T.

The use of the hexagon formula for glucose may also illustrate the readiness with which this sugar may be transformed into derivatives of pyrone. For example,[1] it is known that the growth of Aspergillus oryzæ on koji or steamed rice, or even on glucose, leads to the formation of kojic acid, which is a phenolic alcohol of pyrone. Again, by heating malt one of the products which is formed is maltol, a methyl hydroxypyrone, which is formulated below along with similar types of compounds for comparison.

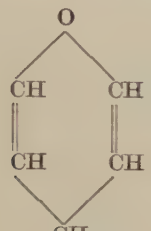


It may therefore be suggested that a genetic relationship may explain the existence together of sugars and of pyran or pyrone nuclei in natural products. Probably it is not without significance that the naturally occurring plant pigments such as the anthocyanins, which occur as glucosides, contain as an essential part of their structure a pyran residue, and that the hydroxyflavones contain the related pyrone group. It is conceivable that this residue is traceable to its origin as a normal sugar, and that these colouring matters of plants are transformation products intimately related in structure to the carbohydrates. The suggestion has also been made that phenols originate through an aldol type of condensation between the potential aldehyde group of an aldohexose and the terminal primary alcohol

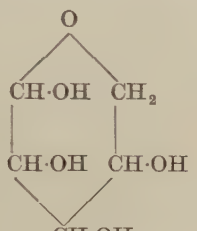
[1] Yabuta, *J. Chem. Soc. Tokyo*, 1916, **37**, 1185, 1234.

group in the sugar chain. This would give a hexahydroxy-cyclohexane of the type of inositol. The elimination of water from the latter leads to the formation of polyhydric phenols.

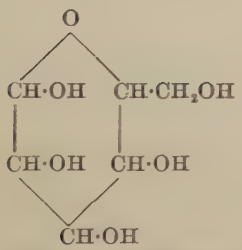
The simplest type of six-atom ring which can be formulated is indeed that of pyran, containing five carbon atoms and one oxygen atom in the ring. Reduction and hydroxylation would theoretically yield a tetrahydrotetrahydroxy-pyran, which can be regarded as the simplest normal sugar, namely a normal pentose. It will be convenient to describe this formula as that of Pyranose.



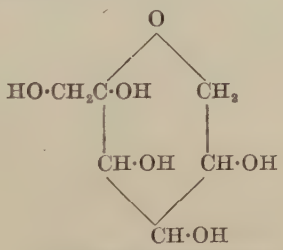
Pyran



A Normal Pentose
(Pyranose (Arabo-, etc.))



A Normal Aldo-hexose
(Gluco (etc.)-pyranose)



A Normal Keto-hexose
(Fructo-pyranose)

By suitable distribution of the hydroxyl groups and hydrogen atoms in such a formula one can arrive at any of the known pentoses. These would conveniently be described by a nomenclature which takes account both of the structural and stereochemical distribution of the addenda. Thus, arabinose, xylose, lyxose and ribose in their normal forms can be correctly described as arabo-pyranose, xylo-pyranose, lyxo-pyranose and ribo-pyranose. Such a terminology would be distinctive and self-explanatory, and if adopted would overcome much of the confusion which has attended sugar nomenclature. The pentosides would naturally be described as methyl xylo-pyranoside, etc., and the related lactone as xylo-pyrone.

If one introduces a side-chain into the above pyranose formula, one arrives at the formulation of a typical hexose. Thus, glucose, galactose and mannose can be considered to be hexapyranoses, and

their configuration would be suitably indicated by the adoption of the terms gluco-pyranose, galacto-pyranose, manno-pyranose.[1] The example of fructose can also be included in this terminology. In this case the side-chain is attached at the same carbon atom as is the reducing hydroxyl. Here again a typical reaction of the fructose derivatives, for example tetramethyl fructose, is the behaviour of the side-chain towards oxidizing agents. Even though the terminal primary alcohol group be methylated, it is transformed, during oxidation, into the carboxyl group of a monobasic acid. This grouping can be eliminated during further oxidation as is shown in formula XXII (page 35), where trimethyl fructuronic acid undergoes conversion into trimethyl arabonolactone. Fructose can therefore be included in the above generalization and may be described as fructo-pyranose.

The proofs of the constitution of the lactones which have so far been given are dependent in each case upon the isolation and recognition of their oxidation or degradation products, and particularly upon the isolation of the appropriate stereoisomeric variety of a trimethoxyglutaric acid, which is identified in the form of its crystalline methylamide and amide. To eliminate any reasonable doubt in the characterization of these final oxidation products, specimens of them have been prepared from the recognized sources for the purpose of comparison.

[1] Goodyear and Haworth, *loc. cit.*

CHAPTER VI

LABILE OR γ-SUGARS, AND THEIR RELATION TO FURAN

(THE FURANOSE SERIES)

More than twenty years after the discovery of the α- and β-methylglucosides, Fischer [1] isolated a third form of methylglucoside which he described as a "γ-form," meaning by the use of the term "γ" that this variety differed materially from the known α- and β-forms. The use of this terminology had, however, no structural or stereochemical significance. Fischer recognized that in this compound he was dealing probably with a substance having a different constitution from the previously recognized forms. The γ-methylglucoside is a liquid, readily hydrolyzable by acids of extreme dilution, and capable of being distilled in a vacuum. It is formed by condensing glucose and methyl alcohol *at room temperature* in the presence of 1% hydrogen chloride.

Two crystalline derivatives of γ-glucose have now been isolated as the α- and β-forms of pentabenzoyl γ-glucose.[2] Their specific rotations ($[\alpha]_D + 58{\cdot}6°$ and $- 52{\cdot}6°$) differ widely from those of the corresponding normal glucose derivatives ($+ 107{\cdot}6°$; $+ 23{\cdot}7°$). The latter values correspond fairly closely to those of α- and β-glucose or -glucopyranose ($+ 110°$; $+ 17{\cdot}5°$), and from these comparisons it may be surmised that, were it possible to isolate the *two* stereoisomeric γ-forms of the free sugar corresponding with α- and β-glucopyranose, the α-form would have a much lower positive rotation than the normal or usual form of α-glucose, whereas the β-form of γ-glucose would be strongly lævorotatory. Again, the above rotations of α- and β-glucose are comparable in their range with the values of the normal α- and β-methylglucosides ($+ 159°$ and $- 34°$), and it would appear that the two stereoisomeric forms of γ-methylglucoside may be expected to possess specific rotations similar in range to the above pentabenzoyl derivatives of γ-glucose, the β-form of γ-methylglucoside having a higher lævorotation than the normal β-methylglucoside. This view is partly confirmed by the isolation [3] of the crystalline carbonate of γ-methylglucoside which has $[\alpha]_D - 64°$.

[1] *Ber.*, 1914, **47**, 1980. [2] Schlubach and Huntenberg, *Ber.*, 1927, **60**, 1487.
[3] Haworth and Porter (*in the press*).

During many years rival formulæ had been advanced to represent the structure of γ-methylglucoside. Meanwhile, the γ-methyl derivatives of most of the other sugars have been prepared and their constitutional study will now be described.

The usual procedure, that of methylation and hydrolysis of either Fischer's γ-methylglucoside or of glucosemonoacetone, leads to the isolation of a liquid form of tetramethyl glucose.[1] This differs widely in its physical and chemical behaviour from the previously recognized crystalline tetramethyl glucose which, it will be recalled, is obtainable either by the methylation of α- and β-methylglucosides or by the hydrolysis of certain methylated disaccharides. Up to the present there has, however, been recorded no authentic case of the occurrence of a γ-glucose residue in any natural product. There is available a simple and precise method by which tetramethyl γ-glucose could be recognized [2] if it should occur as a scission product from methylated natural substances : the crystalline lactone to which it gives rise on oxidation with bromine water furnishes a characteristic derivative by heating with phenylhydrazine, when the phenylhydrazide of the corresponding tetramethyl gluconic acid, m.p. 134–136°, can be isolated. This crystalline lactone has been discussed already on page 19. Its mutarotation or hydration curve has been given, and this also is characteristic and serves to differentiate it clearly from the other tetramethyl gluconolactone (the δ-form) derivable from crystalline tetramethyl glucose.

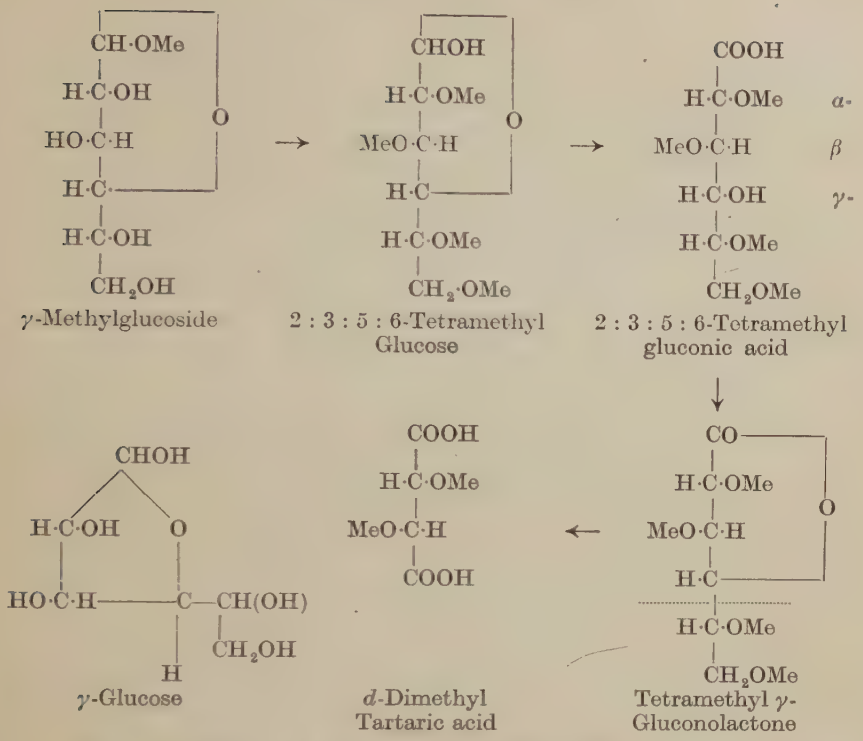
Again, the degradative oxidation of the crystalline γ-form of tetramethyl gluconolactone has been studied.[3] It gives rise on digestion with hot nitric acid to a mixture of *d*-dimethyl tartaric acid and oxalic acid. (See formulæ on opposite page.)

The inference which is definitely drawn from these observations is that this γ-lactone should be formulated as indicated below, scission of the carbon chain having occurred between the fourth and fifth carbon atoms. It follows that the tetramethyl γ-glucose from which the lactone was derived must possess a five-atom ring structure and therefore the orientation of the methoxyl groups is indicated by the formulation of 2 : 3 : 5 : 6-tetramethyl glucose. Since this has in turn been derived by methylation of Fischer's γ-methylglucoside (see across), it follows that the latter must also have a five-atom ring structure. The related free sugar does not appear to be capable of separate existence, but its formula is indicated below for the purpose of comparison with the normal gluco-pyranose.

[1] Irvine, Fyfe and Hogg, *J.*, 1915, 524 ; Micheel and Hess, *Annalen*, 1926, **450**, 21.

[2] Drew, Goodyear and Haworth, *J.*, 1927, 1241 ; Charlton, Haworth and Peat, *J.*, 1926, 100.

[3] Haworth, Hirst and Miller, *loc. cit.*

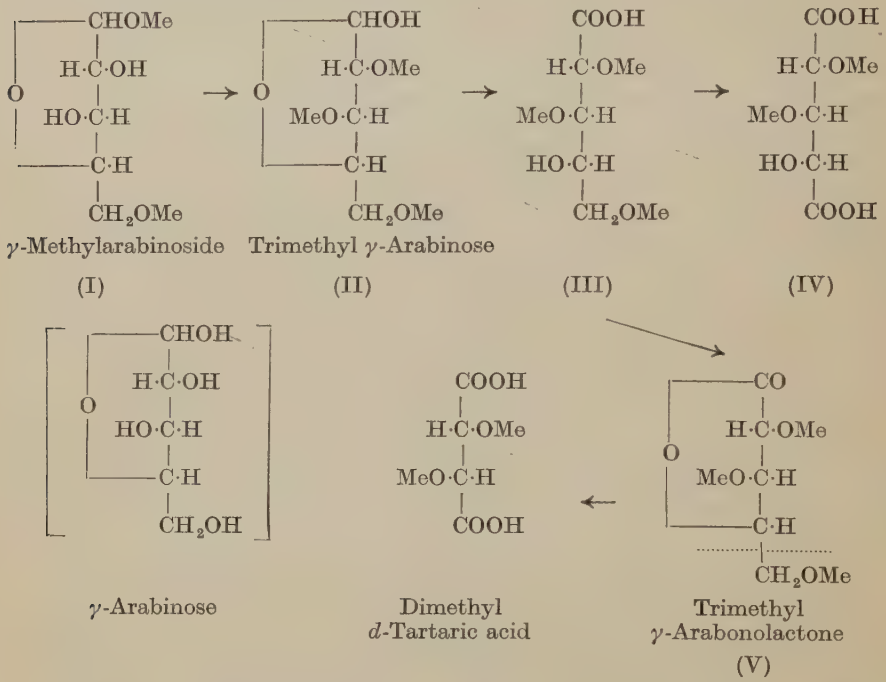


Simple solution of the pentose, *l*-arabinose, in methyl alcohol containing 1 per cent. of hydrogen chloride at ordinary temperatures, has led to the formation [1] of γ-methylarabinoside (I), which is the analogue of Fischer's γ-methylglucoside. Methylation of the γ-arabinoside with methyl sulphate and alkali gave a liquid which, on hydrolysis, led to the isolation of a trimethyl γ-arabinose (II). Direct oxidation of this sugar with dilute nitric acid resulted in the formation of a dimethoxy-hydroxyglutaric acid (IV), but under milder conditions or by the agency of bromine water, the trimethyl γ-arabinose passes to a crystalline trimethyl γ-arabonolactone (V). The mutarotation curve of this product is discussed in the chapter dealing with the hydration of lactones, and it is seen that the rate of hydration contrasts remarkably with that of the structurally isomeric δ-lactone. Degradative oxidation of the trimethyl γ-arabonolactone resulted in the formation [2] of dimethyl *d*-tartaric acid, recognizable through its crystalline methylamide or amide. The latter were identical with specimens prepared by the methylation of tartaric acid.

This experimental observation furnishes a complete proof that the carbon chain of the γ-arabonolactone undergoes scission between

[1] Baker and Haworth, *J.*, 1925, 365.
[2] Haworth, Hirst and Learner, *J.*, 1927, 2432.

the fourth and fifth carbon atoms, inasmuch as the configuration of the dimethyl tartaric acid which was isolated ensures in the case of arabinose that the methoxyl groups are orientated on the right and left within the oxide-ring. This being so, it is possible only to reach the conclusion that the trimethyl γ-arabonolactone has a five-atom ring structure, and so also the trimethyl γ-arabinose and the γ-methyl-arabinoside from which the lactone was derived. These data furnish evidence of the existence in γ-glucose and γ-arabinose derivatives of a different type of ring structure from that occurring in the normal hexosides and pentosides.



A similar experimental study of *γ-derivatives* of galactose,[1] mannose,[2] xylose,[3] lyxose [4] and fructose [5] led to a like conclusion. The detailed experiments with these latter sugars need not be outlined here except in the case of fructose. It may be said at once, however, that the generalization reached is that all γ-sugar derivatives which have so far been studied possess a five-atom ring structure.

[1] Haworth, Ruell and Westgarth, *J.*, 1924, 2468; Haworth and Jones (*in the press*).

[2] Goodyear and Haworth, *J.*, 1927, 3136.

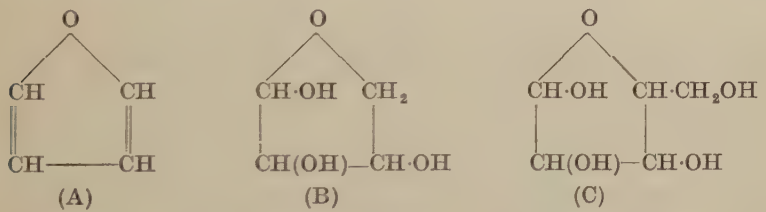
[3] Haworth and Westgarth, *J.*, 1926, 880; Haworth and Porter, *J.*, 1928, 611.

[4] Haworth, Hirst and Smith, *J.*, 1929 (*in the press*).

[5] Haworth, Hirst and Nicholson, *J.*, 1927, 1513; Haworth, Hirst and Learner, *ibid.*, p. 2432; Avery, Haworth and Hirst, *ibid.*, p. 2308.

A discussion of γ-fructose and experiments leading to its characterization and constitution provide a topic of special interest, for the reason that it is this variety of the ketose which alone appears to occur naturally, e.g. in sucrose or cane sugar. This can appropriately and more conveniently be dealt with in the chapter on the disaccharides.

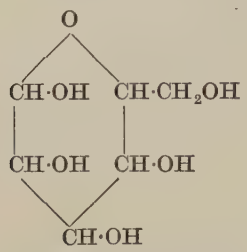
It is possible now to refer to the γ-sugars as a class by themselves and to adopt for these substances a common ring structure. A constitutional study of the derivatives of γ-sugars shows that these are related in structure to the parent form, furan (A), which is the simplest type of the five-atom ring containing four carbon atoms and one oxygen atom. A trihydroxytetrahydro-furan would represent the simplest γ-sugar, namely a tetrose, which is formulated below (B). This may conveniently be given the name of Furanose. By the attachment of suitable side-chains to this formula we can represent all the γ-sugars, beginning with the pentoses and hexoses. The attachment of a $-CH_2OH$ group at the fourth carbon atom of the ring furnishes a general representation of a γ-aldopentose, which might now be called a penta-furanose (C). The configuration of the hydroxyl groups and hydrogens in arabinose, xylose, lyxose and ribose may be suitably indicated by the use of the prefixes arabo-, xylo-, etc., so that the γ-forms of the pentoses would be named arabo-furanose, xylo-furanose, etc.



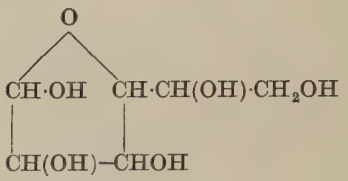
In a similar way the attachment of a lengthened chain to the furanose formula, namely a $-CHOH{\cdot}CH_2OH$ group, enables us to represent the γ-hexoses or hexa-furanoses of the aldose type. The formula below (D) is a general representation of all these hexoses, which can be differentiated according to their configuration by the adoption of the terms gluco-furanose, galacto-furanose, manno-furanose.

The case of γ-fructose differs in an interesting respect, since the furanose model is modified by the attachment of two side-chains, each being $-CH_2OH$. One of these primary alcohol residues is attached at the same carbon atom as the reducing hydroxyl, and the

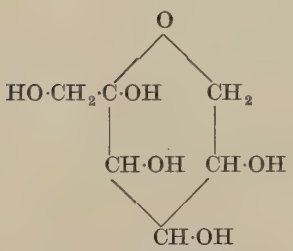
formula for γ-fructose or fructo-furanose is indicated below (E). It is this form of fructose which occurs naturally in sucrose and also in the polysaccharide inulin. The free sugar has never been isolated, since on hydrolysis of sucrose the fructose component passes by isomeric change into the fructo-pyranose or six-atom ring form of ordinary crystalline fructose.



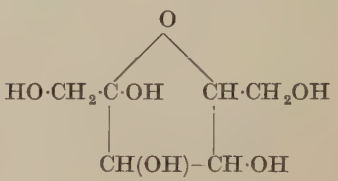
Normal Aldo-hexose
(Gluco (etc.)-pyranose)



γ-Aldo-hexose. (D)
(Gluco (etc.)-furanose)



Normal Keto-hexose
(Fructo-pyranose)



γ-Keto-hexose. (E)
(Fructo-furanose)

Such a reformed nomenclature [1] having a definite relation to the structure and configuration of the sugars may well replace that which has grown up in an empirical way. Earlier on page 39 it has been suggested that the normal sugars, which are formulated as six-atom ring compounds, are related to the parent substance pyran, and these normal sugars are described as pyranoses. The adoption of these suggestions would have the advantage of eliminating many confusing terms as normal, γ, h, amylene-oxide, butylene-oxide, 1 : 5 oxide, 1 : 4 oxide, which in the past have, in many cases, been incorrectly employed in the literature. The terminology may also be extended to the lactones. Thus the γ- and δ-gluconolactones are recognizable as glucopyrone and glucofurone, respectively.

[1] Goodyear and Haworth, *loc. cit.*

CHAPTER VII

ACETONE COMPOUNDS AND SUGAR CARBONATES

Among the many types of condensation which sugars undergo, one of the most interesting is that described by Fischer as the combination of acetone residues at the hydroxyl positions of the sugar. Many of these acetone derivatives are crystalline, and they are readily formed by shaking an acetone suspension of finely divided sugar in the presence of a small amount of hydrogen chloride, zinc chloride or anhydrous copper sulphate. Several of these products can be distilled in a high vacuum.

Their constitution was left undetermined by Fischer. Attempts to apply structural formulæ to the acetone compounds have until recently led to much error and confusion. It may be remarked that had it been found possible to investigate with success their structural formulæ at that time, the correct explanation of the ring systems in sugars would have been anticipated by some twenty years. It seems necessary therefore to revise the constitution of these products in the light of other recently recorded data.

Glucose forms a well-defined diacetone compound (di-*iso*-propylidene glucose).[1] This substance contains only one free hydroxyl group, the remaining four being protected by the two acetone residues. Methylation of this hydroxyl group leads to the formation of a monomethyl glucose-diacetone, and by hydrolysis of the product the acetone residues are eliminated and a monomethyl glucose (crystalline) is formed.[2] These facts illustrate a characteristic behaviour of sugar acetones inasmuch as the acetone groups are stable to alkali but unstable in the presence of dilute acid. The monomethyl glucose is a derivative of normal glucose or gluco-pyranose, since on complete methylation it passes into crystalline 2 : 3 : 4 : 6-tetramethyl glucose.[3] In monomethyl glucose the presence of the methyl group at position 5 in the chain is thereby excluded. It yields on oxidation [4] a monomethyl saccharolactone, and this latter product passes on reduction to a monomethyl *d*-glucuronic acid and not to the *l*-variety. The inference to be drawn from these results is, first, that the methyl group is not in the 6-position, since this position is capable of oxidation

[1] E. Fischer, *loc. cit.* [2] Irvine and Scott, *J.*, 1913, 564.
[3] Anderson, Charlton and Haworth (*in the press*).
[4] Levene and Meyer, *J. Biol. Chem.*, 1923, 57, 317 ; 1924, 60, 173.

to a carboxyl group without loss of a methyl residue from the product, and secondly, that the methyl group cannot be in position 4, since this position is occupied by the lactone linking in the monomethyl *d*-saccharolactone. The only remaining alternatives are positions 2 and 3. Proceeding further with the experiments on the original glucose diacetone, it is found that this gives on hydrolysis a glucose monoacetone which is devoid of action towards Fehling's solution. Consequently the reducing group and one other hydroxyl group of the sugar are occupied by the one remaining acetone residue. Again, the methylation of the glucose monoacetone yields a trimethyl derivative which on hydrolysis passes to a trimethyl glucose. This substance gives a crystalline phenylosazone,[1] and therefore the free positions in the trimethyl glucose, which must also have been the positions previously occupied by the acetone residue in the monoacetone, are positions 1 and 2. Consequently, position 2 is eliminated from consideration as the free hydroxyl group in glucose diacetone. There remains then position 3. Now the trimethyl glucose derived from the glucose monoacetone yields on further methylation a tetramethyl glucose, and this on oxidation is converted to tetramethyl γ-gluconolactone which is crystalline and recognizable also as the crystalline phenylhydrazide. It follows that the trimethyl glucose is a gluco-furanose or γ-sugar, and the position of the 1 : 4 oxide ring in the original glucose-diacetone is revealed. The two acetone residues connect positions 1 and 2, and also 5 and 6, leaving a free hydroxyl group in position 3. This is the formula expressed below (I) for the acetone compound and confirmatory evidence is provided by an alternative study of its hydrazino substitution derivative [2] as well as by a study of the α-fructose-diacetone, which will shortly be considered.

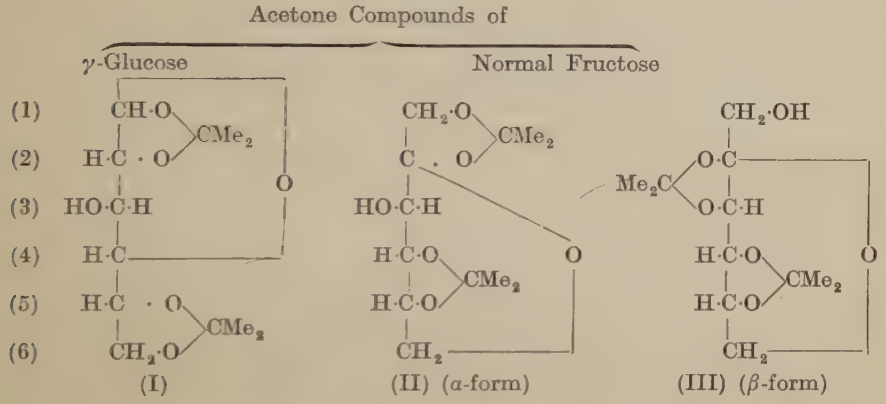
Fructose condenses with acetone under different conditions to give two crystalline products, the α- and β-fructose-diacetones. These are not merely stereoisomerides ; they are structurally different. Each contains a free hydroxyl group and is non-reducing. Methylation of the α-fructose-diacetone gives a crystalline monomethyl derivative,[3] and passes, by removal of the acetone residues with dilute acid, to a crystalline monomethyl fructose. This is a normal fructose derivative or a fructo-pyranose, because on furthur methylation it yields crystalline tetramethyl fructose, the constitution of which is discussed on page 34. Consequently, the methyl residue in monomethyl fructose cannot occupy position 6, the other possibilities open being positions 1, 3, 4 and 5. Now monomethyl fructose can undergo interconversion from fructo-

[1] Anderson, Charlton and Haworth, *loc. cit.*

[2] Freudenberg and Hixon, *Ber.*, 1923, **56**, 2119 ; Freudenberg and Doser, *ibid.*, p. 1243.

[3] Charlton and Haworth (*in the press*). (Compare Irvine and Hynd, *J.*, 1909, 1220.)

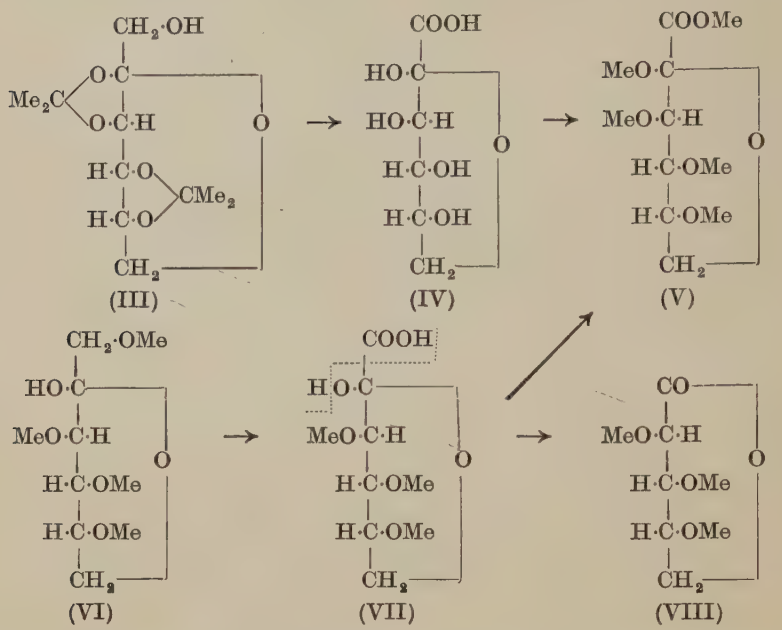
pyranose to a fructo-furanose, that is, it can change [1] to a γ-sugar; therefore position 5 is eliminated as a possibility for the position of the methyl residue. By partial hydrolysis the diacetone yields a fructose-monoacetone which, on methylation and elimination of the remaining acetone residue, gives rise to a trimethyl fructose which is a fructopyranose. Finally, it is shown that the monomethyl fructose yields identically the same osazone as the monomethyl glucose described in the preceding paragraph as arising from glucose diacetone. It follows from this result that both in monomethyl fructose and monomethyl glucose the methyl residue is in position 3, and therefore this represents also the position of the free hydroxyl group in both glucose diacetone (I) and α-fructose diacetone (II). The constitutional formulæ for these compounds are therefore those given below.



The other crystalline diacetone derived from fructose is the β-compound. This substance undergoes oxidation with alkaline permanganate to yield a monobasic acid, the acetone residues remaining intact.[2] It follows then that one of the terminal positions in the diacetone sugar is exposed, that is, position 1 or 6. If the acetone groups be removed from this monobasic acid by the agency of dilute mineral acid and the product (IV) is thereafter completely methylated, there is formed a substance having the constitution of the tetramethyl fructuronic ester (V) indicated below.[3] This gives a crystalline amide and its constitution has been decided on the grounds that crystalline tetramethyl fructose (VI) changes by digestion with nitric acid to a trimethyl fructuronic acid (VII) which, when further methylated, gives the same amide. The trimethyl fructuronic acid has the constitution shown because it passes with acid permanganate into tri-

[1] Allpress, *J.*, 1926, 1720.
[2] Ohle, Koller and Berend, *Ber.*, 1925, 58, 2577.
[3] Anderson and Haworth (*in the press*).

methyl δ-arabonolactone (VIII). The constitution of β-fructose diacetone (III) is therefore finally determined by these results.



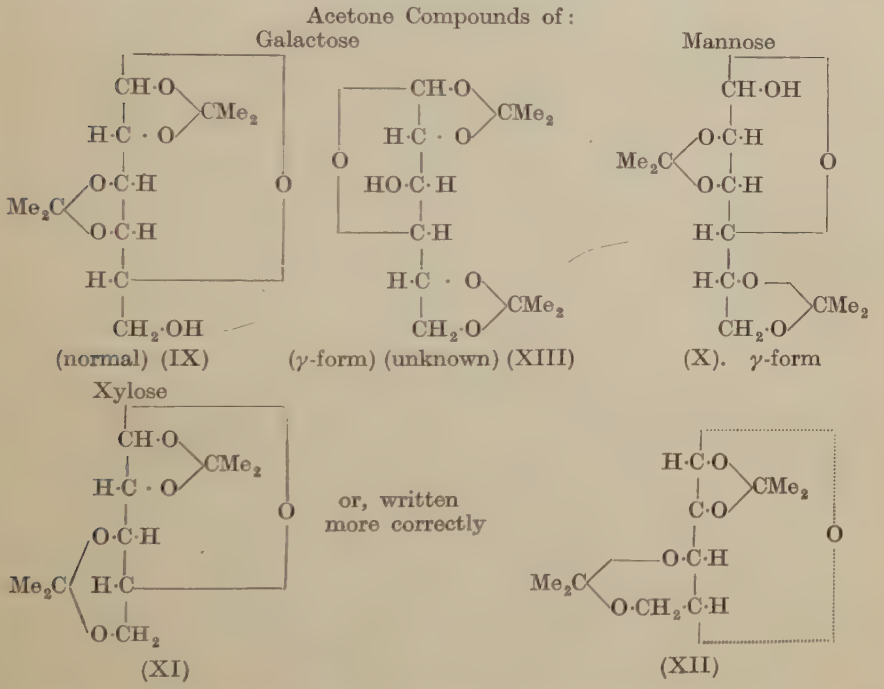
The condensation of galactose with acetone yields a galactose diacetone (IX). This forms a 6-iodohydrin which undergoes reduction to *d*-fucose diacetone, identical with the product obtainable from *d*-fucose, and moreover, *d*-fucose is derivable from it on removal of the acetone residues.[1]

Mannose forms a crystalline diacetone which displays mutarotation, and consequently the reducing group is not protected by either acetone residue.[2] The mannose diacetone can be readily oxidized to give γ-mannonolactone diacetone, identical with that obtainable by condensing acetone and γ-mannonolactone[3] (see page 14). It follows from this and other confirmatory evidence that the constitution of mannose diacetone is indicated by the formula (X) below.

A comparison of the sugar-acetones already discussed reveals the general tendency of acetone to condense with *cis* hydroxyl groups and usually those which are attached to neighbouring carbon atoms, and it would appear that this rule is followed throughout the series. There are few examples in which the condensing hydroxyls at alternate, instead of at contiguous, carbon atoms are involved in the

[1] Freudenberg and Raschig, *Ber.*, 1927, 1633.
[2] Freudenberg and Wolf, *Ber.*, 1925, 300.
[3] Goodyear and Haworth, *loc. cit.*

union with an acetone molecule, and one of these exceptions is the case of xylose-diacetone. Owing to the configuration of xylose, the third and fifth hydroxyl groups are brought into such spatial proximity that the distance is equivalent to that of two *cis* hydroxyl groups associated with neighbouring carbon atoms. The evidence [1] in this case points definitely to the formulation of xylose diacetone as indicated below (XI) and (XII). If in the condensation of xylose with acetone the general tendency, enunciated above, were to be followed, then the oxide-ring system in the sugar would have to adjust itself to a four-atom ring. Otherwise the condition that *cis* hydroxyls associated with contiguous carbon atoms could not hold,



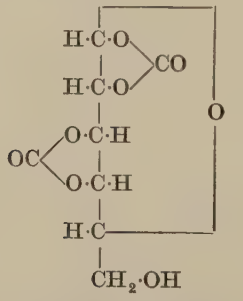
owing to the special configuration which obtains in xylose. It has been demonstrated that the ring system which is actually present in xylose-monoacetone (and therefore presumably in the diacetone) is a five-atom ring as indicated in the formula (XII). The proof is that xylose-diacetone may be hydrolyzed to give the crystalline monoacetone ; and the methylation of the latter, followed by elimination of the remaining acetone residue, gives rise to a dimethyl γ-xylose which yields on oxidation a dimethyl γ-lactone. Methylation of the latter leads to the isolation of trimethyl γ-xylonolactone, iden-

[1] Haworth and Porter, *J.*, 1928, 611.

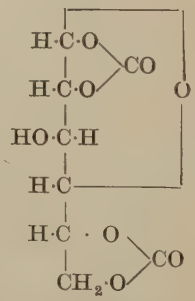
tical with that obtainable from trimethyl γ-xylose (trimethyl xylofuranose).

The conclusion on the structure of acetone condensation products of sugars is reached that acetone residues condense with appropriately situated (structurally and spatially) hydroxyl groups in any sugar regardless of any pre-formed ring system in that sugar. The sugar ring adjusts itself to that position (whether at carbon 4 or 5) left open after the preferential selection of positions of entry is made by the acetone residues. A shift in the position of the oxide-ring occurs in glucose, mannose, and xylose, the diacetone derivatives of which are all substances of the furanose or γ-sugar type. The two fructose-diacetones, and also galactose-diacetone are compounds of the normal six-atom ring or pyranose type. Without violating the rule as to the spatial distribution of hydroxyl groups most favourable to the entry of acetone groups, it is possible that a second galactose-diacetone should also exist (formula XIII) in which the ring system is that of the furanose or five-atom type. But this second form does not appear to have been isolated. There is nothing exceptional in the phenomenon of the displacement of the positions of attachment of the oxide-ring in a sugar due to the union of the sugar with acetone. This indeed is analogous to the displacement which is known to occur when a sugar condenses at room-temperature with methyl alcohol to give, for example, γ-methylglucoside.

A series of new compounds which correspond, structurally, with the acetone series has recently been recorded. These are the sugar-carbonates, which are prepared [1] by condensing sugars with phosgene in pyridine or mildly alkaline solutions. They are described as crystalline substances of the type:



Galactose-carbonate



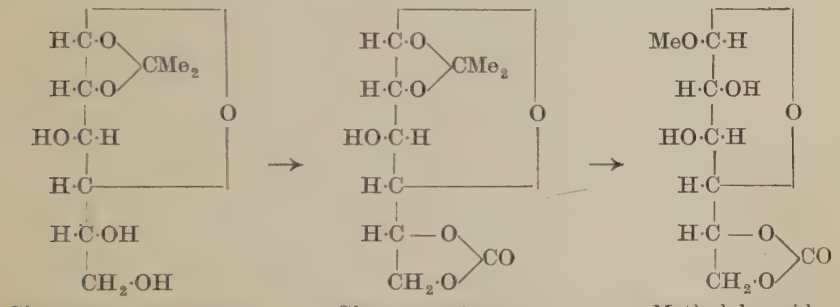
Glucose-carbonate

Interest attaches to the properties of these substances in that, unlike the acetone compounds, they are unstable to alkalies whilst being appreciably more stable to acids than the acetone compounds.

[1] Haworth and Porter (*in the press*).

The disclosure of these properties has rendered possible the preparation of what is probably the first crystalline derivative of γ-methylglucoside, by the following sequence of changes:

Carefully graded hydrolysis of glucose-diacetone leads to the removal of one acetone group and to the isolation of the crystalline glucose-monoacetone. This compound is formed *in situ* when glucose in acetone suspension is treated with phosgene, and condenses to give a glucose-acetone-carbonate which is a crystalline substance having different substituents connecting two pairs of hydroxyls. Digestion with dilute acid in presence of alcohol eliminates the acetone residue and introduces the glucosidic methyl group. These changes are formulated as follows:



The γ-methylglucoside monocarbonate forms large crystals, m.p. 143–145°: $[\alpha]_D$ — 64°. It has a special interest for the reason stated on page 42. The corresponding γ-ethylglucoside monocarbonate is also a crystalline substance, m.p. 163–165°. On the new nomenclature they may be more accurately described as the carbonates of the β-forms of methylglucofuranoside and ethylglucofuranoside.

CHAPTER VIII

THE REDUCING DISACCHARIDES, LACTOSE, MALTOSE, CELLOBIOSE, GENTIOBIOSE, AND MELIBIOSE

The study of the constitution of the bioses is naturally based upon an intimate knowledge of the structure and properties of the individual hexoses and their derivatives. Indeed, this statement applies equally to the study of all the higher members of the sugar series; and it would obviously be impossible to pursue with any hope of success the constitutional investigation of the polysaccharides without the essential preliminary of an exact knowledge of the structure of the disaccharides. Most of the naturally occurring disaccharides, including those which are obtained by simple cleavage of more complex members of the carbohydrate group, are so constituted that they can be regarded as theoretically derivable from the condensation of two hexoses by the elimination of a molecule of water. In most cases the reducing group of one hexose is involved in this type of union with a hydroxyl group of the other hexose component. One may regard this type of linking as analogous in every respect to a glucoside, for example, methylglucoside, except that the methyl group in the latter is replaced by the attachment of a hexose residue. This hexose residue retains in the majority of cases its own reducing group unimpaired, the condensing hydroxyl group being situated lower in the hexose chain. In some bioses, of which sucrose is a conspicuous example, the reducing groups of both hexose components are involved in the biose linking, and these bioses are known as the non-reducing disaccharides. They are devoid of action towards Fehling's solution unless they are made to undergo a preliminary hydrolysis which releases the two reducing groups simultaneously, yielding a mixture of two constituents. On the other hand, the reducing disaccharides are characterized by their capacity to affect Fehling's solution and by the comparative ease with which they form phenylosazones. They also undergo mutarotation changes. This latter group will be considered first.

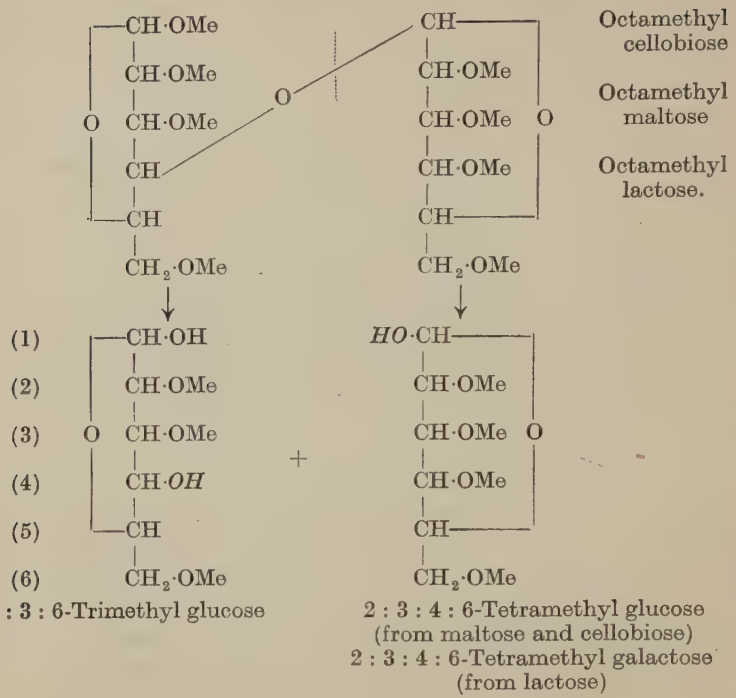
To gain a clear picture of the constitution of the disaccharides, it is necessary to inquire what are the structural or other characteristics which are responsible for the differentiation between, for example, maltose and gentiobiose. It is essential that one should know which of the several hydroxyl groups of one component hexose is linked

with the reducing group of the second component, and it will be seen that several choices are possible. This information on the structural side should then be coupled with the knowledge of configurational relationships. For example, it is not sufficient to know that one glucose component is linked with a second at a definite position unless it be also known whether this component is joined as an α- or a β-glucose residue.

Many of these questions find a complete solution from a study of the methylated disaccharides. There are eight available hydroxyl groups in a dihexose. These may be protected by simple methylation with methyl sulphate and dilute alkali, but the conditions must be so chosen that when the disaccharide is sensitive to acids, as is sucrose, the alkali should always be present in excess. On the other hand, since other disaccharides are adversely affected by alkali, it is necessary to ensure, particularly during the earlier stages of the methylation process, that alkalinity does not develop. These conditions can be controlled by maintaining an excess of methyl sulphate during the addition of dilute alkali solution. Several of the completely methylated disaccharides are crystalline, and all of them can be purified by distillation in a high vacuum. Being methylated, they are now devoid of action towards Fehling's solution, and carefully controlled hydrolysis leads to the development of reducing power and to cleavage into two methylated hexoses. It is now necessary to devise methods by which the two constituents can be separated from this mixture, and further investigation must then be directed to the recognition of the structure of each methylated or partly methylated hexose unit. Evidently, in the act of hydrolysis, one free hydroxyl group in each constituent hexose has been introduced or exposed, and the problem resolves itself into one of deciding at which point in the chain this newly exposed hydroxyl group is situated. The results of some preliminary investigations on the constitution of cellobiose, maltose and lactose may now be given. These are not at this stage decisive, but the complete proof will be developed in the later pages of this chapter.

It is seen that octamethyl cellobiose,[1] octamethyl maltose,[2] and octamethyl lactose [3] all undergo hydrolysis to yield one and the same trimethyl glucose, a crystalline sugar in which the methyl residues have been assigned to positions 2, 3, 6, and the exposed hydroxyl group is here provisionally represented at the fourth carbon atom of the chain. This unit represents the reducing component in the three disaccharides. The non-reducing component is recognizable

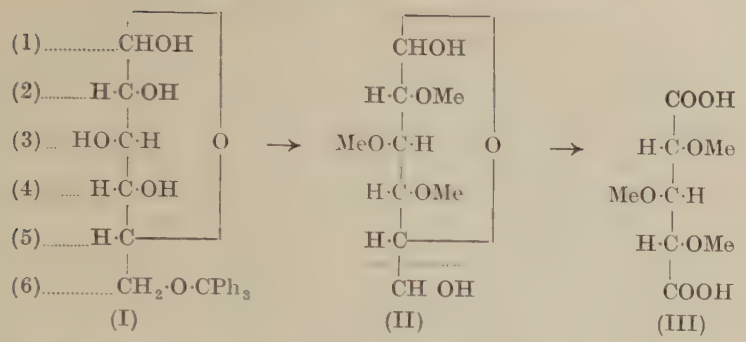
[1] Haworth and Hirst, *J.*, 1921, 193.
[2] Irvine and Black, *J.*, 1926, 862 ; Cooper, Haworth and Peat, *ibid.*, p. 876.
[3] Haworth and Leitch, *J.*, 1918, 188.



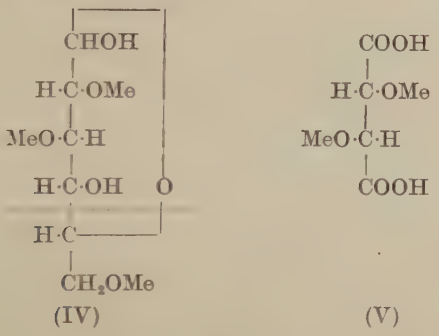
as a crystalline tetramethyl sugar in each case. From methylated maltose and cellobiose this is indeed found to be 2 ; 3 : 4 : 6-tetramethyl glucose (or gluco-pyranose), whilst from lactose the corresponding 2 : 3 : 4 : 6-tetramethyl galactose (or galacto-pyranose) is isolated.

The allocation of the positions of the substituent methyl groups in 2 : 3 : 6-trimethyl glucose is founded on the following observations.[1] This and two other trimethyl glucoses (2 : 3 : 4-, and 2 : 4 : 6-) are known, and all three have these properties in common : (*a*) they are destitute of the capacity to form an osazone ; (*b*) they undergo further methylation to give the same crystalline form of tetramethyl glucose—2 : 3 : 4 : 6-tetramethyl glucopyranose. By theory there is no other trimethyl glucose possible which can conform to these specifications. It is evident from (*a*) that position 2 is substituted by a methyl group, and there are available three other positions for the remaining two methyl groups. Now the 2 : 3 : 4-trimethyl glucose has been prepared by a method which affords evidence of its constitution. The 6-triphenylmethyl glucose (I) has been shown to have the constitution here allocated to it, and by methylation and subsequent hydrolysis it gives 2 : 3 : 4-trimethyl glucose (II) which is oxidized by nitric acid to xylo-trimethoxy glutaric acid (III).

[1] Haworth, Learner and Long (*in the press*).



On the other hand, the 2 : 3 : 6-trimethyl glucose (IV) is attacked by the oxidizing agent at the junction of the 4th and 5th carbons of the chain to give *d*-dimethyl tartaric acid (V). The 2 : 4 : 6-trimethyl glucose gives neither (III) nor (V) on oxidation.

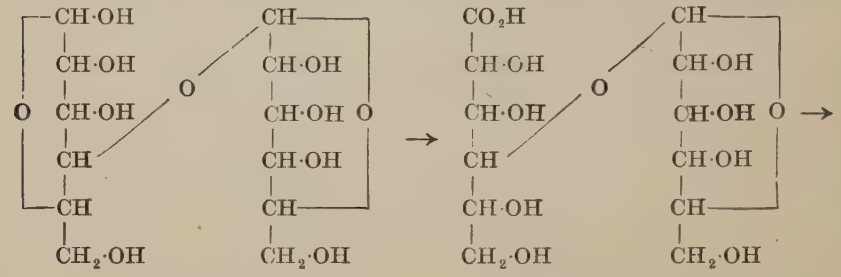


The above preliminary results relating to cellobiose, maltose and lactose require confirmation on the structural side in one important particular. It is evident that the isolation of a 2 : 3 : 6-trimethyl glucose might reasonably be interpreted in a manner which would lead to dubiety. It may be suggested that the biose linking had previously engaged a hydroxyl group at the fifth carbon atom, and that the group at the fourth carbon atom was appropriated in ring formation. This would mean that the ring structure of one unit in the disaccharide would be that of a gluco-furanose or γ-sugar. Now none of the three disaccharides displays the properties of a γ-sugar, but it is of vital importance that so large an issue should not be left undecided. The solution to this problem has been found in the following way.[1]

The reducing group in maltose is converted by oxidation with bromine water into a carboxyl group, with consequent opening of the oxide-ring. This product, analogous to gluconic acid, is known as maltobionic acid, and if the provisional structure indicated already

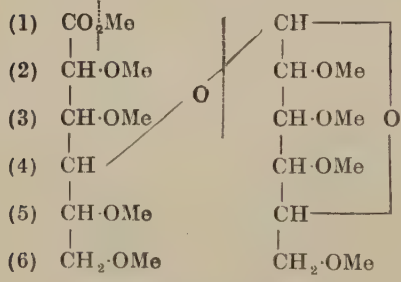
[1] Haworth and Peat, *J.*, 1926, 3094.

for maltose be the correct one, then the point of union of the biose linking is at position 4 in the reducing component of maltose. Consequently the formula for maltose and for maltobionic acid will be those indicated below. Complete methylation of maltobionic acid introduces nine methyl residues, one of these being an ester grouping. Isolation of this product has been followed by its complete hydrolysis, and this led to the recognition of two substances. One of these is the easily recognizable 2 : 3 : 4 : 6-tetramethyl glucose, the crystalline sugar which has already been discussed, and the second is a tetramethyl gluconic acid which, on heating, passes into a crystalline lactone. The latter was recognized from its properties and and from its crystalline phenylhydrazide as 2 : 3 : 5 : 6-tetramethyl

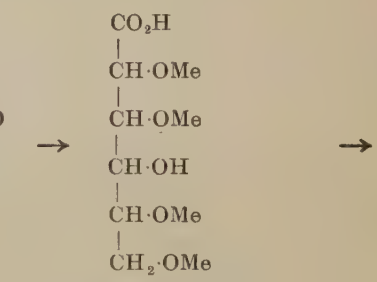


Maltose

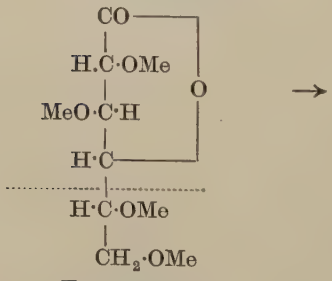
Maltobionic acid



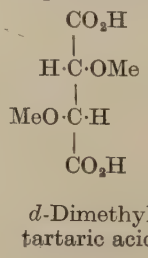
Methylated maltobionic acid



2 : 3 : 5 : 6-Tetramethyl gluconic acid



Tetramethyl γ-glucono-lactone



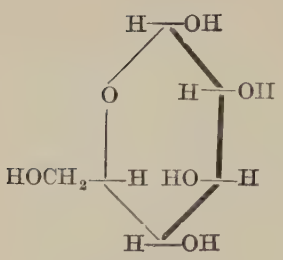
d-Dimethyl tartaric acid

γ-gluconolactone which, it will be remembered (pages 42, 43), undergoes degradative oxidation to a *d*-dimethyl tartaric acid. It is evident, then, that a γ-lactone can only be formed from a gluconic acid in which a hydroxyl at the fourth carbon atom is exposed, and this exposed hydroxyl is obviously the one which had previously participated in the biose linking with the non-reducing component in maltose. The only alternative is thus seen to be an impossible one, namely, that the biose linking is at position 5 in the reducing hexose chain, since this would have led to the isolation and recognition of a δ-gluconolactone, and this is not the case.

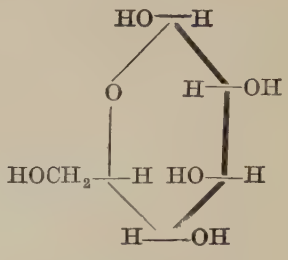
The experimental proofs which are here developed have been applied in analogous researches to the cases also of cellobiose [1] and lactose.[2] The final conclusion is reached that the three disaccharides, maltose, cellobiose and lactose, have a common structure and are only distinguishable by their having a different configuration. These constitutional points are indicated in the formulæ given below, which indicate also the stereochemical relationships of the three disaccharides. The recognition of the configuration at the biose junction is based on the behaviour of the bioses with enzymes. Thus, maltose has the α-configuration inasmuch as this sugar is completely hydrolyzed by maltase, which is the specific enzyme for the hydrolysis of α-methylglucoside. Cellobiose is hydrolyzed by emulsin which is specific for β-methylglucoside ; whilst lactose undergoes scission with lactase, the specific enzyme for β-galactosides. Confirmation of these spatial relationships is furnished by chemical means also (see page 65).

The following structural formulæ suffice to indicate the interrelationships of the three disaccharides, since the configuration at the biose junction is correctly represented :

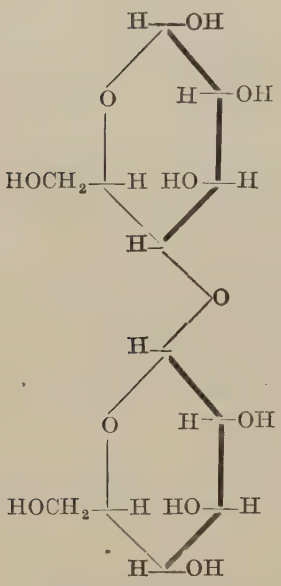
[1] Haworth, Long and Plant, *J.*, 1927, 2809.
[2] Haworth and Long, *ibid.*, 544.



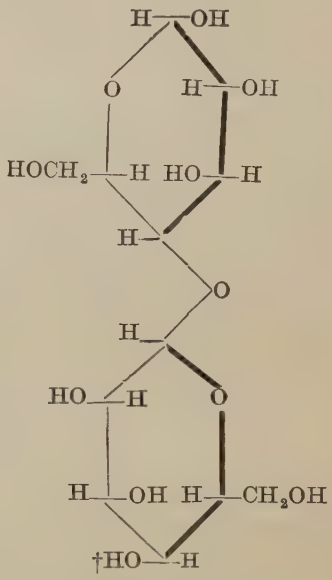
α-Glucopyranose



β-Glucopyranose



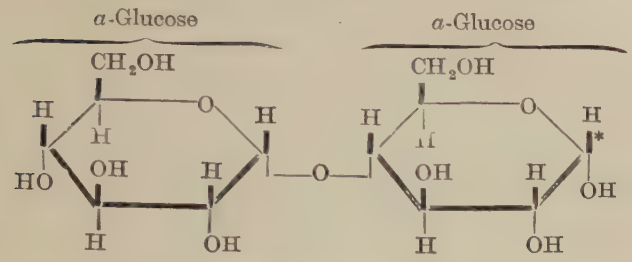
Maltose (α-form)

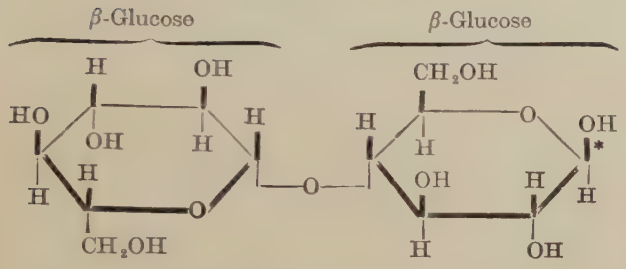


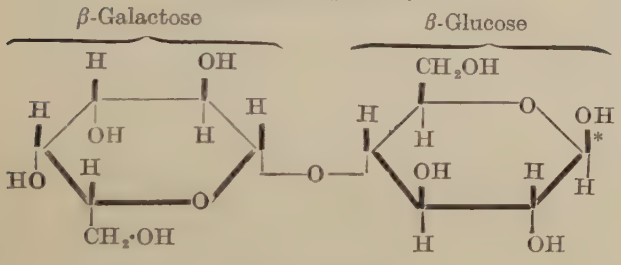
Cellobiose (α-form)

[The cellobiose formula applies also to *Lactose* when the configuration at position † in the second residue is modified to conform with β-galactose (see below).]

A careful study of the atom models of maltose and cellobiose is essential for the understanding of the stereochemical relationships in the polysaccharides. For the purpose of reference, and as an aid to the construction of such models, alternative methods of writing the structural and stereochemical formulæ of the three disaccharides are appended. The following serve also to emphasize the essential difference in the configuration of maltose and cellobiose at the biose junction. The free reducing group of each disaccharide is indicated by an asterisk *.



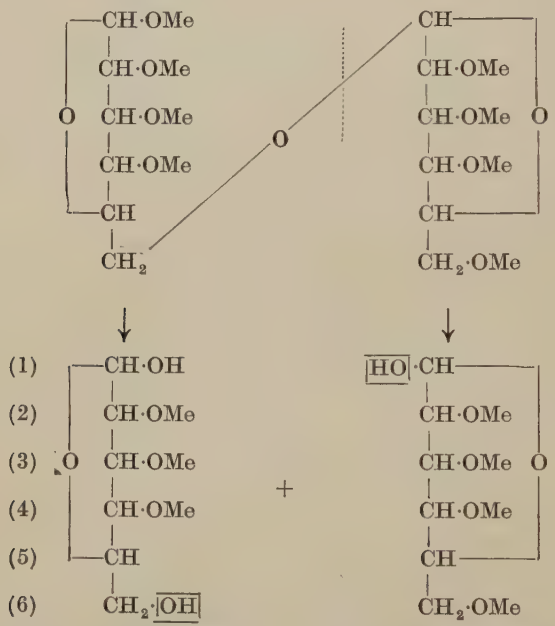




A similar mode of inquiry may be extended to the disaccharides, gentiobiose and melibiose. Here it is found that the completely methylated disaccharides, which are crystalline, undergo hydrolytic cleavage to give two methylated components, one of which is recognizable as 2 : 3 : 4-trimethyl glucose, which forms a crystalline methyl-glucoside. The remaining component in the case of gentiobiose is 2 : 3 : 4 : 6-tetramethyl glucose (tetramethyl-gluco-pyranose) and the corresponding unit from the octamethyl melibose is the crystalline 2 : 3 : 4 : 6-tetramethyl galactose (tetramethyl-galacto-pyranose). Evidently these two bioses differ from those already studied inasmuch as the reducing group of one hexose unit is joined with a terminal hydroxyl in the side-chain of the reducing hexose component.[1]

[1] Haworth and Wylam, *J.*, 1923, 3120; Haworth, Hirst and Ruell, *ibid.*, 3125; Charlton, Haworth and Hickinbottom, *J.*, 1927, 1527; Haworth, Loach and Long, *ibid.*, 3146.

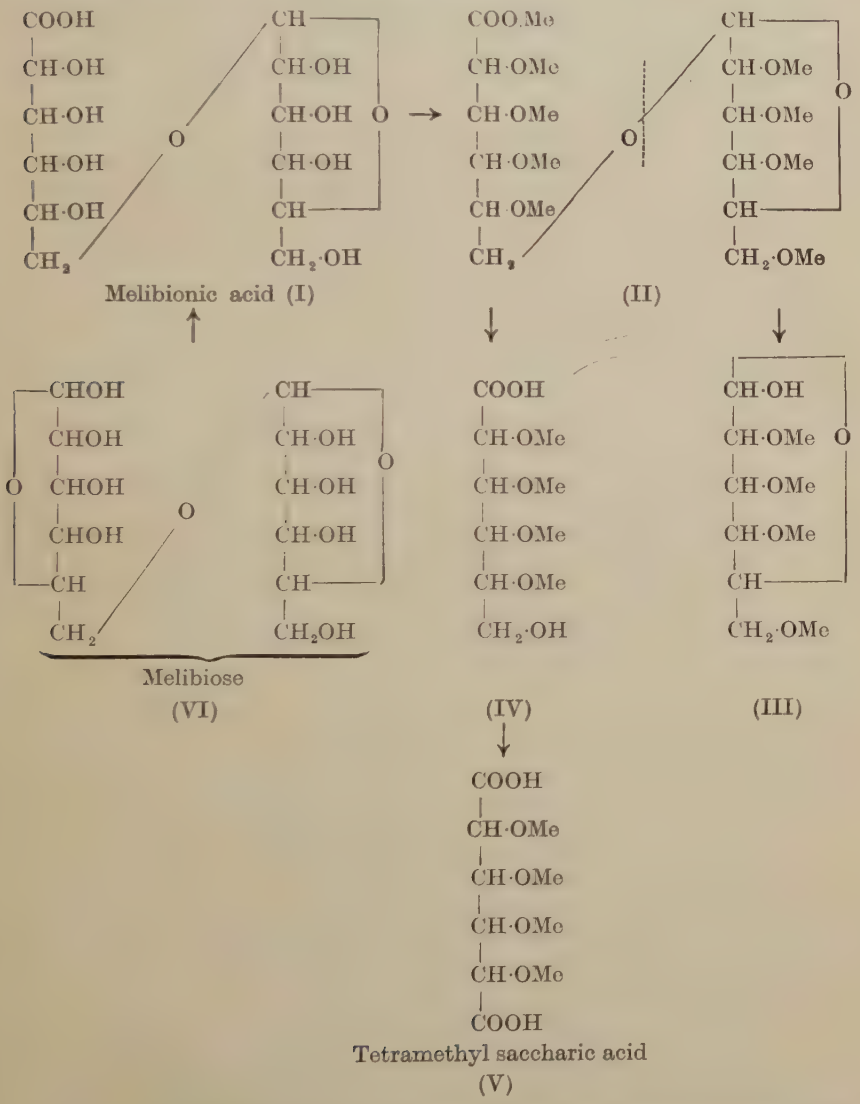
This distinguishing feature is of special interest, inasmuch as gentiobiose occurs as the sugar residue in amygdalin (which has been synthesized from gentiobiose) [1] and possibly in other glucosides, and this biose is also formed from the trisaccharide, gentianose, by fermentation. By an analogous procedure melibiose is obtained by yeast fermentation from the natural trisaccharide, raffinose. Hence it seems that whilst the polysaccharides such as cellulose and starch are the sources of one structural type of disaccharide, represented by cellobiose and maltose, yet the trisaccharides and glucosides, on the other hand, represent a different class of substances in which the hexoses are united through the intermediary of a side-chain or sixth hydroxyl position.



Further confirmation of these structural formulæ for gentiobiose and melibiose is furnished by the structural study [2] of the monobasic acids derived from these bioses, for example, melibionic acid (I). This substance is obtained by the oxidation of melibiose with bromine water, and, on methylation, yields methyl octamethyl melibionate (II). Hydrolysis of this compound gives rise to two cleavage

[1] Campbell and Haworth, *J.*, 1924, 1337.
[2] Haworth, Loach and Long, *loc. cit.*

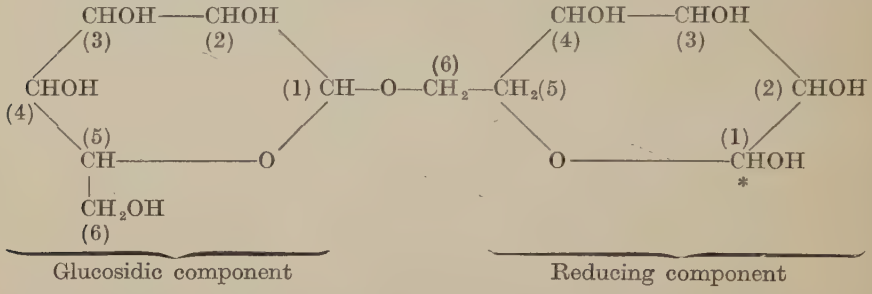
products, one of them recognizable as the crystalline 2 : 3 : 4 : 6-tetramethyl galactose (III) and the remaining one as 2 : 3 : 4 : 5-tetramethyl gluconic acid (IV), which yields a crystalline ester, but apparently not a lactone. Further oxidation of the acid or ester with nitric acid leads to the formation of tetramethyl saccharic acid (V), recognizable through its crystalline methyl ester and its crystalline methylamide and amide, and identical with a specimen prepared by the direct methylation of saccharic acid.



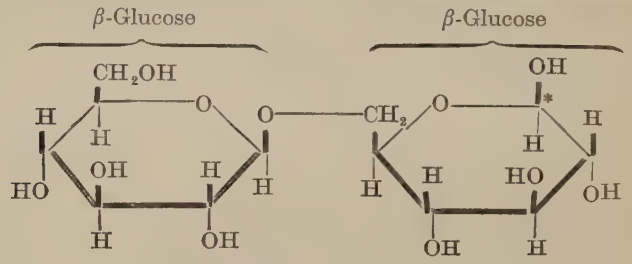
There can therefore be no doubt that the introduction of the

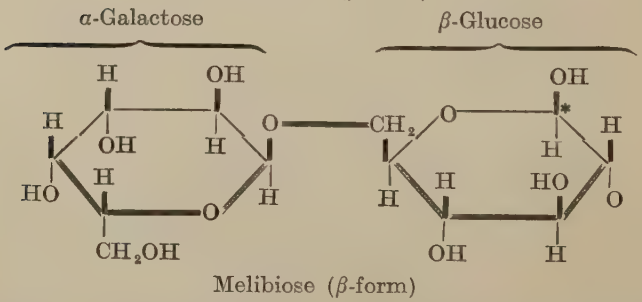
second carboxyl group by this latter oxidation, proceeded through the pre-existing — CH_2OH group in the tetramethyl gluconic acid. Hence it follows that this cleavage product of methylated melibionic acid contains a terminal primary alcohol group, which was exposed only on cleavage at the bionic junction, and therefore the position of the biose linking in melibiose (VI) is seen to be through the side-chain attached to the ring of the reducing hexose in the disaccharide.

Melibiose and gentiobiose are therefore represented by the following general formula :



The complete constitutional formulæ of the two disaccharides are as follows ; the reducing group is indicated by the asterisk *.



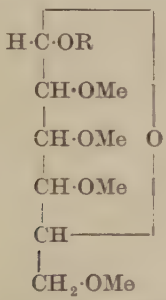


Gentiobiose is hydrolyzed by emulsin which is specific for β-methylglucoside, and thus the β-configuration is given to the grouping at the biose junction. The corresponding configuration of the group-

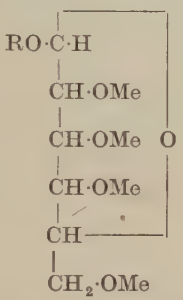
ing of the biose junction in melibiose is considered to be that of α-galactose for the following reason. The specific rotations of the methyl esters of four octamethyl bionic acids are recorded below:

	$[\alpha]_D$	Equilibrium value attained on hydrolytic cleavage
(1) Methyl octamethylmaltobionate	+ 121°	→ + 54·9°
(2) Methyl octamethylmelibionate	+ 106°	→ + 64°
(3) Methyl octamethylcellobionate	+ 5°	→ + 55°
(4) Methyl octamethyl-lactobionate	+ 34°	→ + 77·2°

These products are analogous to the methylglucosides and methylgalactosides and may be represented by the general formulæ:



α-Glucoside or galactoside



β-Glucoside or galactoside

The residue R in the above formulæ is a methylated gluconic acid component in each case. On the analogy of the wide differences in specific rotations displayed by α- and β-forms of methylglucosides and galactosides, the stereochemical forms of the above esters, Nos. 1 to 4, may be inferred. The following figures are given for comparison:

	$[\alpha]_D$
α-Methylglucoside	+ 158°
α-Methylgalactoside	+ 193°
β-Methylglucoside	− 34°
β-Methylgalactoside	− 1°

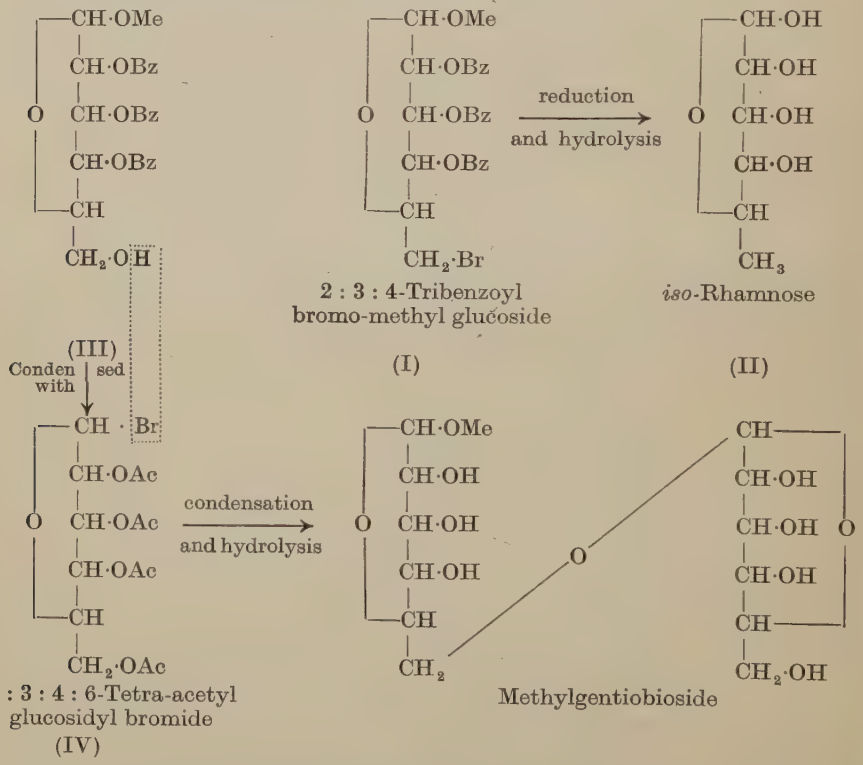
Significance attaches to the small magnitude of rotation of the esters (3) and (4) as compared with the high values of the esters (1) and (2). The former correspond to the β-glucoside and galactoside and the latter to the α-glucoside and galactoside. However, it is observed that following the hydrolytic cleavage of Nos. (3) and (4), the rotations are enhanced to a higher value due to the mutarotation of the liberated β-sugar to the equilibrium of α- and β-forms. On the other hand, on hydrolysis of the esters Nos. (1) and (2) their

F

rotations decline to a lower value since the newly liberated α-sugar falls to an equilibrium value.

On these grounds [1] Nos. (1) and (2) are α-derivatives of a sugar and Nos. (3) and (4) are β-derivatives. Maltose is thus a glucose-α-glucoside; melibiose is a glucose-α-galactoside; cellobiose is a glucose-β-glucoside; and lactose is a glucose-β-galactoside.

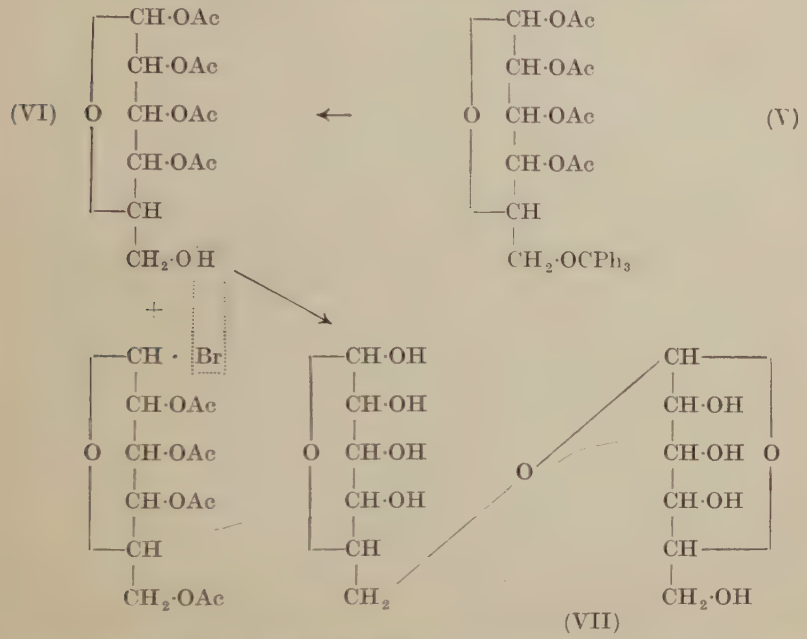
A synthesis of gentiobiose has been achieved [2] which furnishes a structural and stereochemical proof of its constitution, and this is in agreement with that already established by the experiments just described. The starting point is 6-triphenylmethyl methylglucoside which gives the tribenzoyl-bromo-methylglucoside shown below (I). Its structure is determined by the fact that it gives *iso*-rhamnose (II) on reduction and hydrolysis. The initial substance also yields 2 : 3 : 4-tribenzoyl methylglucoside (III) in which the terminal or sixth group carries a hydroxyl. This is condensed with tetra-acetyl glucosidyl bromide (IV) which is known to lead to β-glucosides by condensation with alcohols, and the product on elimination of the acyl groups gives rise to methylgentiobioside.



[1] Haworth, Loach and Long, *loc. cit.*

[2] B. Helferich, Klein and Schäfer, *Annalen*, 1926, **447**, 19.

Utilizing now 1 : 2 : 3 : 4-tetra-acetyl triphenylmethylglucose (V), the same workers succeeded in obtaining 1 : 2 : 3 : 4-tetra-acetyl glucose (VI) which they condensed similarly with the above tetra-acetyl glucosidyl bromide and, by elimination of the acetyl residues from their condensation product, isolated gentiobiose (VII).



The validity of the synthesis [1] as a proof of constitution depends on the determination of the structure of the tetra-acetyl glucose (VI). This was established by the conversion of the triphenylmethyl derivative (V) into Fischer's acetodibromoglucose, and this in turn gives *iso*-rhamnose on reduction and hydrolysis. The structure of the tetra-acetyl glucose (VI) is also supported by other and independent work.[2] An analogous method of synthesis also supports the formula and configuration here assigned to melibiose.[3]

[1] B. Helferich and Klein, *Annalen*, 1926, **450**, 225.
[2] Oldham, *J.*, 1925, 2840.
[3] B. Helferich, *Annalen*, 1928, **465**, 170.

CHAPTER IX

SUCROSE (CANE SUGAR), AND THE TRISACCHARIDES, RAFFINOSE AND GENTIANOSE

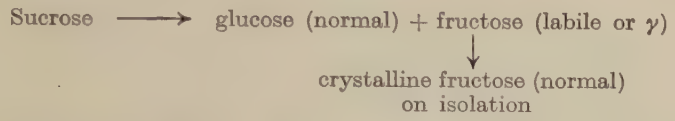
The problem of the constitution of sucrose has presented many difficulties, both of a theoretical and experimental nature. As is well known, sucrose having $[\alpha]_D + 66{\cdot}5°$ undergoes hydrolysis to invert-sugar having $[\alpha]_D - 20{\cdot}5°$. From the invert-sugar the ordinary crystalline glucose and fructose can be isolated. It would seem therefore from these experiments that the components which are united in the most important of the disaccharides are the ordinary forms of glucose and fructose. That this is not the case was first suggested by the experimental work of Haworth and Law.[1] They demonstrated that octamethyl sucrose having $[\alpha]_D + 66{\cdot}5°$ underwent complete hydrolysis without inversion of sign to a mixture of methylated glucose and fructose having $[\alpha]_D + 56{\cdot}5°$. They were able to show that the methylated fructose fragment was probably a γ-sugar and that here in sucrose there had arisen the first example of the occurrence of a γ-sugar residue in a natural product.

One of the unexplained observations in the earlier work of Purdie and Paul [2] had some relation to this problem, in that these authors, in endeavouring to prepare tetramethyl fructose by the methylation of methylfructoside, had obtained a by-product which was evidently a tetramethyl fructose but had a low positive rotation. Haworth and Law re-interpreted this result in the sense that Purdie and Paul had probably a tetramethyl γ-fructose present as an impurity. That this form of fructose could also be obtained from methylated sucrose was a new observation.

The next step in the inquiry into the constitution of sucrose was the isolation by Haworth [3] of the pure fructose component in the form of tetramethyl γ-fructose. This was a liquid having $[\alpha]_D + 31{\cdot}7°$, and its isolation as a hydrolysis product of heptamethyl sucrose was facilitated by reason of the lower volatility of the remaining glucose

[1] *J.*, 1916, 1314.
[2] *J.*, 1907, 289.
[3] Haworth, *J.*, 1920, 199. (In view of recent claims to priority for this work, it seems necessary to make these facts clear.)

component which was present in the mixture as trimethyl glucose. Haworth and Law explained that the sequence of changes in the hydrolysis of ordinary unsubstituted sucrose was to be represented in the following way :

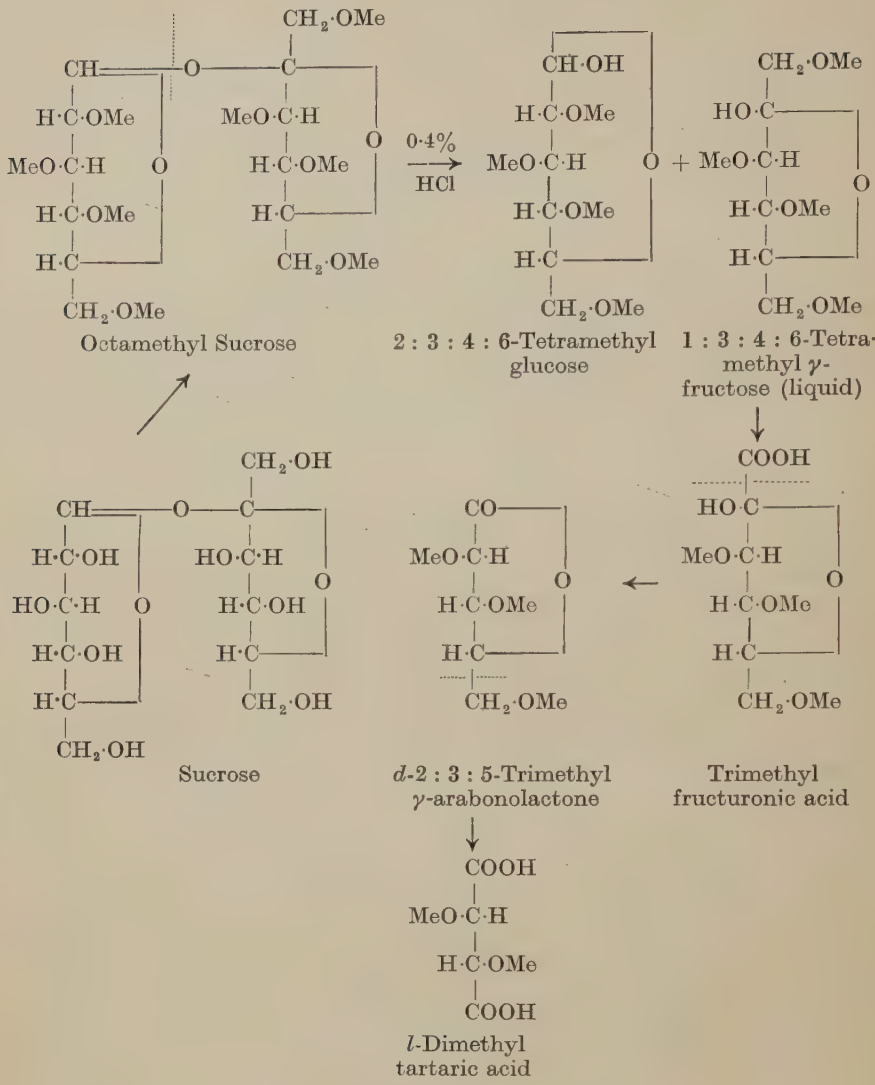


The meaning of this is, that it is apparently not possible under present experimental conditions to isolate the labile variety of fructose which is first formed on hydrolysis of sucrose by dilute acids. This labile variety passes, immediately on its liberation from its union with glucose, into ordinary crystalline fructose by a displacement of the position of its oxide ring.

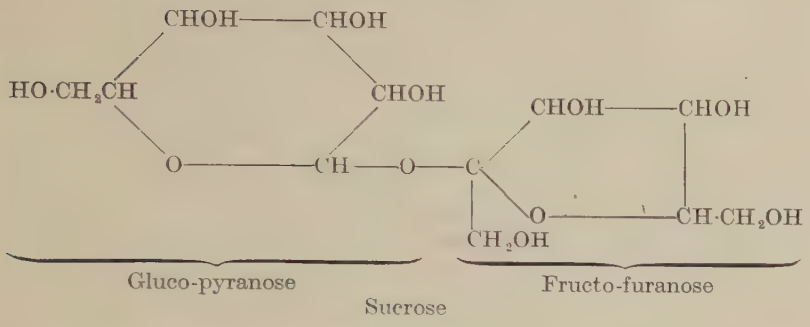
The next step was to determine the constitution of the fructose component of sucrose. For this purpose the tetramethyl γ-fructose isolated from methylated sucrose was examined. Oxidation with nitric acid led to the formation of a liquid trimethyl fructuronic acid by oxidation of a terminal group, with consequent loss of a methoxyl residue. The latter acid showed a reducing action with Fehling's solution, but this property vanished on protecting the reducing group by a methyl residue. The resulting tetramethyl fructuronic ester formed a crystalline amide, and the analysis of this product gave a clue to the molecular formula of the oxidation product. Again, the trimethyl fructuronic acid was found to be capable of easy degradation by the agency of permanganate in the presence of dilute sulphuric acid, giving rise to a crystalline 2 : 3 : 5-trimethyl *d*-arabonolactone. This substance had a specific rotation which was equal in magnitude but opposite in sign to that of the *l*-variety previously isolated by Baker and Haworth [1] in their work on *l*-trimethyl γ-arabinose. Moreover, with both the *d*- and *l*-forms of the trimethyl γ-arabonolactone, further degradation was promoted by oxidation with nitric acid. The product in the case of the lactone which had originated from the fructose component of sucrose was *l*-dimethyl tartaric acid whilst the corresponding *d*-acid was obtained from the *d*-lactone. Each gave a crystalline methylamide, and the *l*-acid was found to be identical in every respect with a specimen prepared synthetically from *l*-tartaric acid. The sequence of changes involved in these oxidations and transformations is indicated below : [2]

[1] *J.*, 1925, 365.

[2] Avery, Haworth and Hirst, *J.*, 1927, 2308 ; Haworth, Hirst and Nicholson, *ibid.*, p. 1513 ; Haworth, Hirst and Learner, *ibid.*, p. 2432.



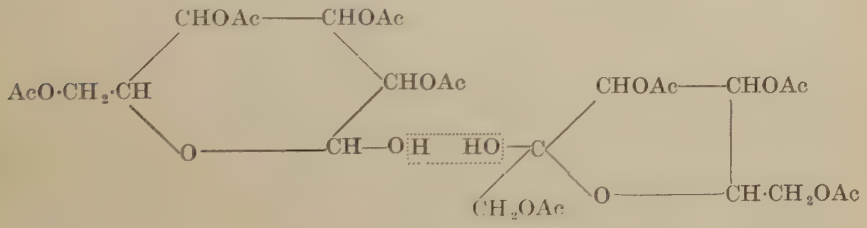
It will be seen that since the remaining component (other than γ-fructose) in the sucrose molecule is represented by 2 : 3 : 4 : 6-tetramethyl glucose (tetramethyl glucopyranose), the final inference which can be drawn is that octamethyl sucrose is to be represented by the formula above, and that this is in turn derivable only from a disaccharide having the constitution which is here applied to sucrose. The constitutional formula for the most important of the disaccharides is therefore indicated by the mutual linking of fructo-furanose with gluco-pyranose through their reducing groups.



Sucrose

It is not yet known whether the α- or β-form of either hexose is involved in the linking at the biose junction.

A. Pictet and Vogel[1] have recently published details of a synthesis of sucrose which takes account of the constitution already elucidated in the course of the preceding experiments. Realizing that the fructose member must be a dextrorotatory or γ-form of fructose, they succeeded in isolating from the acetylation products of fructose a tetra-acetyl fructose in a liquid form which was dextrorotatory. The crystalline tetra-acetyl fructose, which is a fructo-pyranose, is produced simultaneously in the acetylation change. This was effectively separated from the dextrorotatory variety and the latter was then condensed in chloroform solution with tetra-acetyl gluco-pyranose in the presence of phosphoric oxide. In this way the crystalline octa-acetyl sucrose was isolated, and by removal of the acetyl residues the isolation of the genuine synthetic sucrose was at last achieved. This synthesis does not elucidate, however, the stereochemical nature of the grouping at the biose junction.



Trisaccharides, Raffinose, and Gentianose

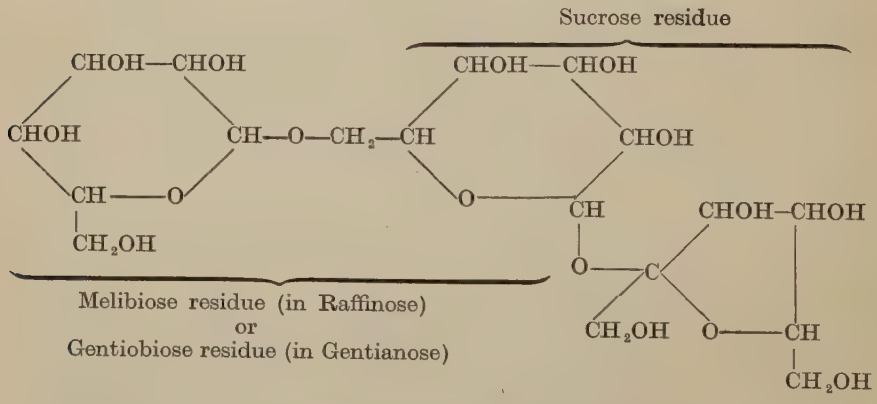
Accompanying sucrose in many natural products, there occurs a crystalline trisaccharide which is devoid of action on Fehling's solution and is known as raffinose. This sugar gives on hydrolysis a

[1] *Helv. Chim. Acta*, 1928, **11**, 436.

mixture of equal quantities of fructose, glucose, and galactose. The mode of union of these three hexoses in the trisaccharide has been determined in the following way.[1]

Methylation of raffinose introduces eleven methyl groups into the trisaccharide, and this on hydrolysis yields tetramethyl γ-fructose (fructo-furanose), trimethyl glucose, and tetramethyl galactose. Chloroform extraction of the hydrolyzed solution of the methylated trisaccharide removes the fructose and galactose components, and on evaporation of the aqueous portion there is obtained the trimethyl glucose, which gives a crystalline derivative in the form of 2 : 3 : 4-trimethyl β-methylglucoside. The constitution of the latter is determined by its simultaneous hydrolysis and oxidation to a 2 : 3 : 4-trimethyl gluconolactone and by degradation with nitric acid to a xylo-trimethoxyglutaric acid. The chloroform extract gives on evaporation a mixture of methylated hexoses from which, by digestion with aniline, a crystalline 2 : 3 : 4 : 6-tetramethyl galactose anilide was separated. This was identical with the methylated galactose anilide obtainable from ordinary methylgalactoside. The fructose component was represented by tetramethyl γ-fructose (fructo-furanose) identical with that obtained from methylated sucrose.

Moreover, earlier work had demonstrated that raffinose could be hydrolyzed by enzymes in two ways: by the action of emulsin, sucrose and galactose could be isolated, whilst in contact with invertase raffinose is converted into melibiose and fructose. The structural formula to be assigned to raffinose must therefore take account of these facts, and the combined experimental results lead to the adoption of the following constitution:



Another important trisaccharide known as gentianose may be

[1] Haworth, Hirst and Ruell, *J.*, 1923, 3125; Charlton, Haworth and Hickinbottom, *J.*, 1927, 1527; Haworth, Loach and Long, *ibid.*, p. 3146.

extracted from gentian root. This sugar is devoid of action towards Fehling's solution and yields, with dilute acids, a mixture of fructose and glucose. By the agency of emulsin it may be converted into a mixture of sucrose and glucose; or alternatively, invertase effects the transformation into fructose and gentiobiose. Since the constitutional formulæ of both these derived disaccharides, sucrose and gentiobiose, are known,[1] it follows that the constitution to be assigned to gentianose is that given above, with the necessary modifications which are indicated.

[1] *Vide supra.*

CHAPTER X

THE POLYSACCHARIDES

General. In considering the group of the polysaccharides we enter a field of constitutional inquiry in which there are many speculations and numerous rival formulæ. Conflicting views are debated, but no finality is to be expected in the present state of knowledge. The time is probably not distant when greater harmony of opinion will prevail and opposing conceptions which reveal only parts of truth will be reconciled by a comprehensive generalization. Meanwhile the elucidation of the ring structure of sugars has given a new impetus to constitutional study. The allocation of the hexagon formula to glucose has provided new interpretations of the experimental evidence bearing on the constitution of the polysaccharides. These are reflected in the examination of the crystal structures of cellulose and chitin by X-ray methods.

If, as is widely accepted, some of the disaccharides and trisaccharides are representative hydrolytic fragments of the polysaccharides, then the structural formulæ which have now been determined for these simpler prototypes must lend valuable aid to the study of the wider problem.

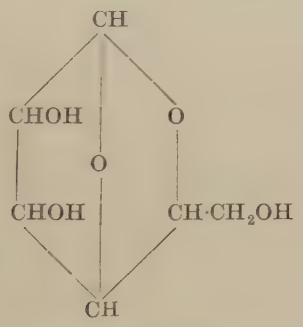
Polysaccharides furnish few derivatives, and the most fertile method of procedure is to study the many scission products to which they give rise. Evidently the phenomenon of polymerization plays some part in the formation of the complex members of this group, and much discussion has arisen on the interpretation of the factors which are at play during polymerization.

The discussion centres upon two main ideas. The first of these is that a polymerizing unit of definite constitution undergoes molecular association by the exercise of residual or auxiliary valencies, and that when the associated product or polymeride is subjected to a variety of external conditions it may revert to the original polymerizing unit. Obviously the adherents of this school endeavour to trace or isolate the constitutional unit of each polysaccharide which may be responsible for these important transformations.[1] If such a unit could be identified and prepared synthetically it would clearly be possible to obtain in the laboratory synthetic specimens of the several

[1] K. Hess and C. Trogus, *Ber.*, 1928, **61**, 1982; Irvine and Robertson, *J.*, 1926, 1488; Pringsheim, *Ber.*, 1926, **59**, 3008.

polysaccharides. The study of a confessedly recondite problem would then be facilitated, and the elaboration of complex molecules which are at present the peculiar contribution of the plant would be imitated by relatively simple experimental means. Several of the sugar derivatives are found to undergo polymerization with exceptional ease, and examples of this kind of change will be given in subsequent paragraphs. It may be said, however, that up to the present there has been no authentic case of the formation of a natural polysaccharide by any such procedure.

A second school accepts the relevant premises of the first, but differs as to their interpretation and final conclusion. It seems clear that in plant life polysaccharides are built up from hexose units by a mechanism which might equally well be that of condensation or polymerization. The condensation of hexoses to more complex sugars such as trisaccharides has frequently been achieved in the laboratory, but it is not suggested that such a union occurs through the exercise of other than ordinary primary valencies. The γ-sugars are prone to undergo self-condensation. It has been suggested that γ-glucose (gluco-furanose) may, in its reversion to glucopyranose, pass through the stage of the 1 : 4, 1 : 5 anhydride



1 : 4-anhydroglucopyranose

which may then polymerize. There appears to be no marked difference in the mode of linking of hexose units in polysaccharides from that which is operative in di- and tri-saccharides. It is considered doubtful whether the scission of a polysaccharide to simpler compounds involves anything more than the severance and union with another grouping of a primary valency link.[1] The theory that starch or cellulose is a diglucose-anhydride or a triglucose-anhydride, which is endowed with a capacity for association involving the interplay of residual valencies, is a hypothesis which is so far

[1] Haworth and Learner, *J.*, 1928, 621 ; Freudenberg and Braun, *Annalen*, 1928, **460**, 288 ; Haworth, *Helv. Chim. Acta*, 1928, **11**, 548.

unsupported by the behaviour of polysaccharides. The latter are known to suffer degradation in such a way that disaccharides and monosaccharides are formed along with the so-called dextrins, which are still complex. By heating starch with glycerol or by the vacuum distillation of starch [1] a variety of anhydro-sugars is formed, but in the process of formation it seems that the essential structural character of the polysaccharides has been masked by secondary changes. A depolymerized unit such as β-glucosan (1 : 5–1 : 6 glucose-anhydride), truly, undergoes polymerization at a high temperature in the presence of zinc chloride to complex bodies,[2] but none of these has so far been shown to have a clear relation to starch. The difficulty of effecting this breakdown process and the use of drastic methods to effect it would suggest that if only residual valency forces have to be overcome, then the experimental methods selected to achieve it have nothing to differentiate them from those which are commonly applied to overcome the presumably stronger primary valency forces.

An experimental achievement which is worthy of more serious consideration is the isolation by Schardinger and by Pringsheim of a series of well-defined amyloses $(C_6H_{10}O_5)_x$ by the action of *B. macerans* on starch. Examples of these compounds are di-amylose, tri-amylose, tetra-amylose, hexa-amylose, and octa-amylose, some of which are crystalline substances. Karrer [3] has shown that these products are closely related to maltose. The action of acetyl bromide on tetra-amylose is seen to lead initially to di-amylose followed by the quantitative formation of aceto-bromo-maltose. Polymerized products which have been subjected to careful study suggest definitely that the type of linking of the units in such polymerides is through the exercise of ordinary valency. It seems difficult to explain why a diglucose- or triglucose-anhydride should be presumed to be endowed with a capacity for the exercise of residual valency. It is probably true that the *mechanism* by which the plant effects the synthesis of polysaccharides is by the utilization of the smallest sugar units, but the idea of mechanism of formation should not be confused with the conception of structure.

Certain observations, of Hess [4] and his co-workers and of Pringsheim,[5] necessitate careful consideration on the former view. A redetermination of the molecular weight of cellulose diacetate in acetic acid solution, from which dissolved oxygen is eliminated by

[1] A. Pictet and Sarasin, *Helv. Chim. Acta*, 1918, **1**, 87.

[2] A. Pictet and Ross, *Helv. Chim. Acta*, 1922, **5**, 876; A. Pictet and J. Pictet, *Helv. Chim. Acta*, 1921, **4**, 788.

[3] *Helv. Chim. Acta*, 1921, **4**, 169, 679.

[4] *Annalen*, 1926, **448**, 99. Compare *Annalen*, 1926, **450**, 29–66; 1927, **455**, 81, 205; 1927, **456**, 38, 55; *Ber.*, 1928, **61**, 1982.

[5] *Annalen*, 1926, **450**, 255; *Ber.*, 1926, **59**, 3008.

a vacuum, gives a mean value corresponding closely with that required by a diacetate of a glucose anhydride, $C_6H_{10}O_5$. This value remains constant for some days and then gradually increases to infinity. The material isolated from the solutions is identical with the initial substance and undergoes the same sequence of changes when redissolved.

This behaviour extends also to crystalline cellulose triacetate and to lichenin triacetate. It is reported that the acetates of cellulose which are recovered from the acetic acid solution are convertible into pure cellulose and again into the acetates without apparent change in structure. It has also been reported that when cellulose triacetate or lichenin triacetate is heated in the presence of naphthalene at 235°, the molecular weight corresponds with that of a triacetate of glucose-anhydride. This diminution in complexity is accompanied by increased solubility in acetone and by a falling viscosity, although there is said to be no change in rotation. Elimination of the acetyl groups discloses an anhydro-glucose which, from cellulose acetate, is termed cellosan, and is soluble in water. Its molecular weight corresponds with the simple formula $C_6H_{10}O_5$. A similar procedure has led to the recognition of lichosan, which is said also to be a glucose anhydride.

Cellosan and lichosan are regarded by the above authors as the elementary units of cellulose and lichenin. On this view these polysaccharides are to be regarded as the products of associated glucose-anhydrides, a hypothesis which was advanced by Hess on the basis of his experimental work on the nature of the copper-ammine solutions of cellulose. Cellosan appears to undergo re-association in solution without any accompanying change in the specific rotation. Again, the rotations of lichenin and lichosan are said to be identical. The re-association of these elementary units seems therefore to call into play no re-adjustment of intra-molecular valency, and if the interpretation of the experimental data be accepted, these features provide abundant ground for speculation as to the nature of the forces promoting molecular aggregation.

Molecular weight determinations of a specimen of inulin triacetate, prepared by the acetylation of inulin with acetic anhydride in presence of pyridine, furnish a value which corresponds with a hexa-acetyl difructose-anhydride.[1] It is thus suggested that the polymerizing unit of inulin is a difructose-anhydride, $C_{12}H_{20}O_{10}$. Hydrolysis of the acetyl derivative is said to lead to regenerated inulin, although there are difficulties in identifying the product in this case. On the other hand, the molecular weight of inulin dissolved in liquid ammonia is said to correspond with the simpler unit $(C_6H_{10}O_5)_2$. Trimethyl

[1] M. Bergmann and Knehe, *Annalen*, 1926, **449**, 302.

inulin dissolved in phenol shows a molecular weight of nine such units,[1] a result which is in agreement with that determined for inulin triacetate in naphthalene, glacial acetic acid, and phenol. If the inulin triacetate derived *via* pyridine is determined cryoscopically in air-free acetic acid, the molecular weight varies with time and corresponds with the presence of one fructose-anhydride unit, increasing up to infinity.

These conflicting data point either to the capacity of polysaccharides to assume remarkably simple forms in certain solvents or else, and this is more probable, to the unreliability of the methods which have here been applied in molecular weight determinations. On the former hypothesis it would seem that the view of Hess, namely that polysaccharides exist as associated hexose-anhydride units, is well supported. Hess advanced a formula [2] for cellulose, part of which may be written as follows, the expression being repeated so as to comprise thirty or even sixty such units of $C_6H_{10}O_5$:—

$$\begin{matrix}\leftarrow\cdots O : \\ \leftarrow\cdots O :\end{matrix}\left[C_6H_7(OH)\right]\begin{matrix}OH \\ OH\end{matrix}\leftarrow O :\left[C_6H_7(OH)\right]\begin{matrix}OH \\ OH\end{matrix}\leftarrow O :\left[C_6H_7(OH)\right]\begin{matrix}OH\leftarrow\cdots \\ OH\leftarrow\cdots\end{matrix}$$

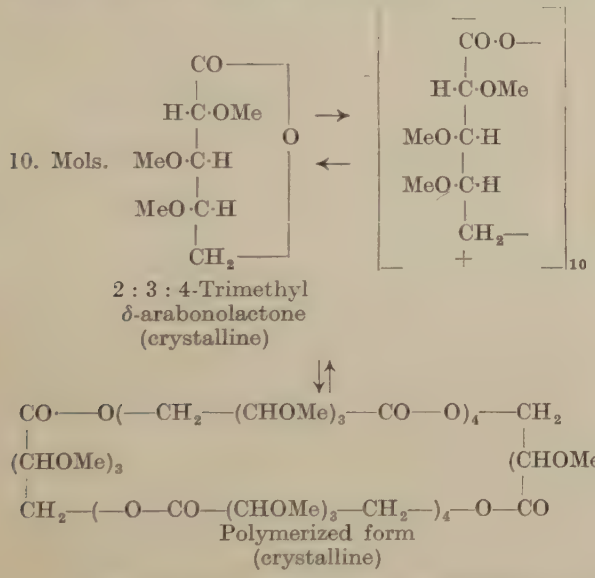
Adopting the view that association involves the operation of co-ordinate co-valencies on the model of Sidgwick's suggestions,[3] it would seem that the oxygens of the two oxide-rings in a glucose-anhydride are co-ordinated with the hydrogens of two hydroxyl groups. The suggestion is indeed attractive, but it is difficult to reconcile the persistence of these co-ordinate linkings in those cases where the hydroxyl groups have been replaced by methoxyl. On this ground alone such a hypothesis becomes difficult of acceptance, and moreover Hess seems disposed now to adopt the hypothesis that the structural unit of cellulose is a biosan which he claims to have isolated. By methylation cellulose is deprived of its capacity to form the copper-ammine. Freudenberg has prepared from trimethyl cellulose the 2 : 3 : 6–trimethyl glucose-anhydride having the rings in positions 1 : 4 and 1 : 5, but it is found that this product is a low-boiling liquid which shows no tendency to polymerize and seems to possess no property in common with the original trimethyl cellulose.

In this connection there may be mentioned an example of a crystalline polymerizing unit which changes spontaneously into a crystalline polymeride which, on heating, passes back again into the simpler polymerizing unit.[4] The substance indicated is 2 : 3 : 4-trimethyl arabonolactone. The crystalline polymeride has ten times the molecular weight of the original lactone, and both solubility and specific

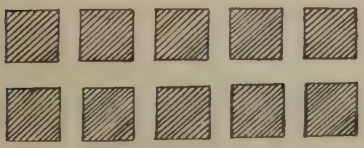
[1] Karrer and Lang, *Helv. Chim. Acta*, 1921, **4**, 249.
[2] *Annalen*, 1923, **435**, 116.
[3] *Electronic Theory of Valency* (Clarendon Press), 1927.
[4] Drew and Haworth, *J.*, 1927, 775.

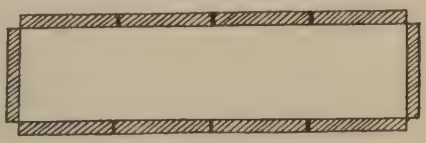
rotation are materially changed in the polymerized state. The properties of the polymeride suggest that here the cyclic form of the lactone undergoes spontaneous scission followed by condensation to a closed chain in which each pentonic acid unit is linked with its neighbour through the exercise of ordinary co-valencies. Its capacity to revert to the simple state may be thus attributed to the electronic condition associated with the presence of the $-\overset{\overset{\cdot\cdot}{O}}{\overset{\|}{C}}-O-CH_2-$ linking, as well as to the special configuration and the crystalline grouping.

This raises the question of the possible existence or development in a polymerized unit of centres of electro-valency corresponding to a di-pole. The rearrangement of grouping involved by the development of such a system may explain its breakdown to the simpler product.



The inference that, in the polymeride, the lactone ring has opened to a chain as shown, is supported by X-ray data.[1] These indicate that the singularity in spatial disposition of one OMe group in the lactone has given place to uniform distribution of the three OMe groups in space.


10 mols. lactone


one mol. polymeride

[1] J. Young (*in the press*).

The spacing of the ten molecules of lactone in the crystal may be indicated diagrammatically by the shaded square figures. The lactone rings open and become elongated to a chain, the ends of which unite to give a looped or cyclic figure for the polymeride. Surrounding the spirally arranged units of the chain, the methoxy groups are symmetrically accommodated in a spacing bounded by an equilateral triangle which may be drawn in a plane perpendicular to that of the chain.

Inulin.—One of the most promising lines of approach to the study of molecular complexity in polysaccharides may prove to be through the investigation of inulin. Inulin is soluble in warm water from which it may be recovered by cooling. The apparent molecular weight in boiling water shows a continuous diminution over a considerable period of time, and the reducing power of the solution rises as the molecular weight decreases.[1] Thus the value of about 4,000 attained after 4 minutes is diminished to half after 28 minutes. There seems to be little, if any, reversion to the original state on cooling the solution. This change to a simpler body is evidently accompanied by hydrolysis. It would appear that the initial complex of inulin contains not fewer than 20 or 24 anhydrofructose units. A breakdown to simpler molecules occurs with remarkable readiness, even in boiling water. This change is promoted by admitting carbon dioxide to an aqueous solution of inulin. Under these conditions a product is formed containing six or eight anhydrofructose residues in the molecule, representing an intermediate stage in the hydrolysis of inulin to fructose. This product corresponds to the dextrins derivable from starch and may be provisionally named " inulin lævulin." It is a white solid displaying considerable reducing power towards Fehling's solution.

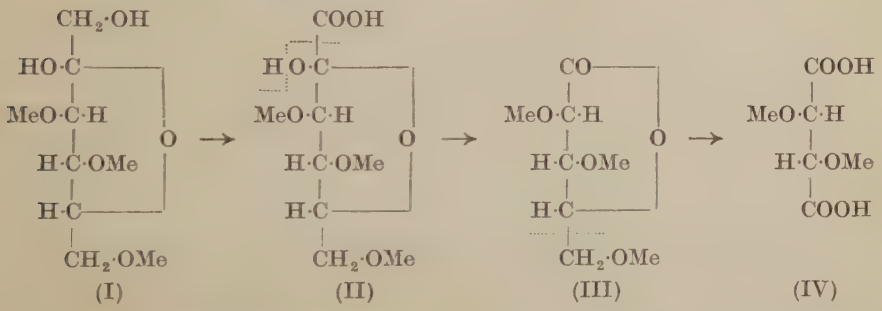
The mode of linking of fructose residues in inulin has been determined.[2] Thus, on methylation with methyl sulphate in alkali solution, inulin yields a trimethyl derivative. This is presumably a partial hydrolysis product of the original polysaccharide which has undergone methylation. But the determination of the positions of the junctions of contiguous fructose residues in such a product gives a sufficient clue to the mode of linking in the initial inulin itself. The " trimethyl inulin " is a white powder, insoluble in water but soluble in benzene, acetone, or alcohol, and devoid of action towards Fehling's solution.

Hydrolysis of this product yielded a trimethyl fructose which gave a crystalline osazone. Evidently the carbon positions 1 and 2 carried free hydroxyl groups, whilst the methyl residues were attached

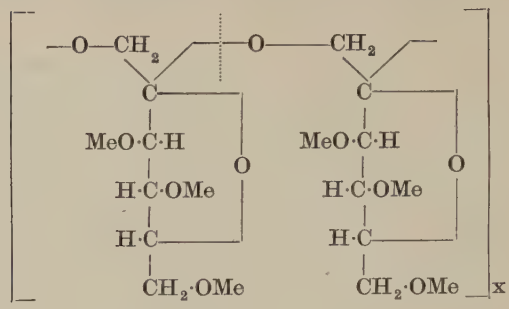
[1] Drew and Haworth, *J.*, 1928, 2690.
[2] Haworth and Learner, *J.*, 1928, 619.

at three of the available positions, 3, 4, 5, 6 in the chain. The precise allocation of these positions was rendered possible by the following experimental methods which admit only of the interpretation that the methyl groups in the trimethyl fructose are attached at positions 3, 4, 6; whilst the oxide ring connects positions 2 and 5. The substance is thus a derivative of γ-fructose or fructo-furanose.

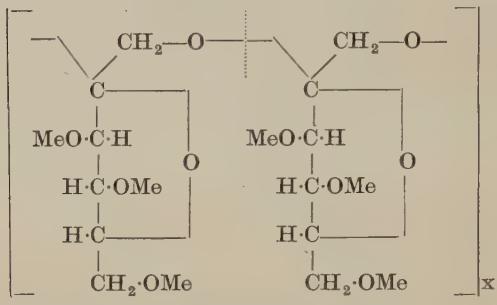
Oxidation of the trimethyl γ-fructose (I) with nitric acid yielded a monobasic acid (II) which is trimethyl fructo-furonic acid. This on methylation gave a tetramethyl ester which formed a crystalline amide. Further oxidation of the trimethyl acid (II) with acid permanganate led to the isolation of the crystalline *d*-2 : 3 : 5-trimethyl γ-arabonolactone (III) (see page 69) which was further degraded by nitric acid to give *l*-dimethyl tartaric acid (IV). The latter has been recognized through its crystalline methylamide which is identical with a specimen prepared from *l*-tartaric acid.



It is now possible to consider how the trimethyl fructofuranose (I) may be joined as contiguous units in the trimethyl inulin derivative from which it was obtained by hydrolytic cleavage. The most symmetrical arrangement of linkings which accommodates the above experimental facts is the junction of pairs at the reducing group (2) of one residue with the terminal group (1) of another such residue. Here stereochemical considerations arise, and it is uncertain whether α- or β-fructofuranose is to be represented, but each of these alternatives is given below.

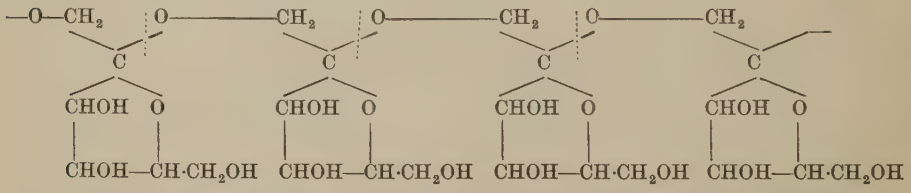


or



A less symmetrical arrangement of the pairs of units is to regard the junctions as occurring alternately through two reducing hydroxyls (positions 2) and through two primary alcohol residues (positions 1). This arrangement seems, on the whole, doubtful in view of the properties of inulin. It is seen that hydrolytic cleavage along the dotted line in the above formulæ would lead to the formation of the trimethyl fructofuranose (I).

Knowledge of the position of the linkings in contiguous units of anhydrofructose in inulin has thus been achieved, and the constitution may be written as follows, the number of units being more than six and probably as great as twenty-four.



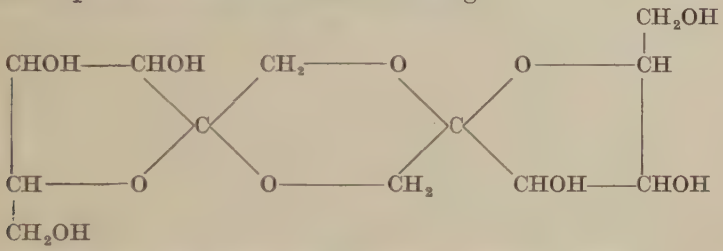
Complete hydrolysis of inulin with aqueous oxalic acid furnishes crystalline fructose by cleavage at the positions shown by dotted lines, but this scission is accompanied by a profound change in the ring structure at the moment of liberation of the reducing sugar units. The fructo-furanose (γ-fructose) at once reverts to the six-atom ring form of fructo-pyranose.

The mere shortening of the conjugated molecule by breakdown

at intermediate positions does not promote the above inter-conversion, since the reducing group (2) is not liberated throughout the whole length of the chain and the shortened molecule preserves its five-atom ring structure just as does γ-methylfructoside.

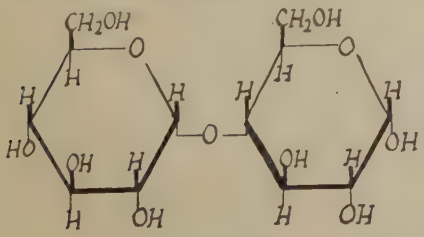
Evidently the scission of the inulin chain occurs with remarkable readiness in the neighbourhood of the fructosidic oxygen, perhaps more readily than with any other polysaccharide. A careful study of the conditions effecting this change in inulin may well be rewarded by a wider knowledge of all the polysaccharides.

It has been suggested that triacetyl inulin is a simple compound which can be formulated as a triacetyl difructose-anhydride. If this should prove to be the case, then the molecular unit in this acetylated product would be the following :

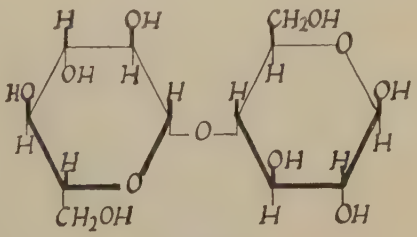


It cannot be too much emphasized, however, that the presence of traces of mineral matter or electrolytes in polysaccharides seems to have a marked, and as yet unexplained, bearing on their properties. The suggestion has been made, that the difference between starch and glycogen may be attributed only to the higher mineral content of the latter ; also Samec suggests that the differing phosphate content of amylose and amylopectin is accountable for the properties of these two constituents of the starch granule.

Starch and Cellulose.—In the chemical evidence bearing on the constitution of starch and cellulose the outstanding facts are that over 80 per cent. of starch can be converted into maltose and that, on a conservative estimate, 40–50 per cent. of cellulose can be transformed into cellobiose. It has been established (see chapter on disaccharides) that the structural formulæ of maltose and cellobiose are the following :



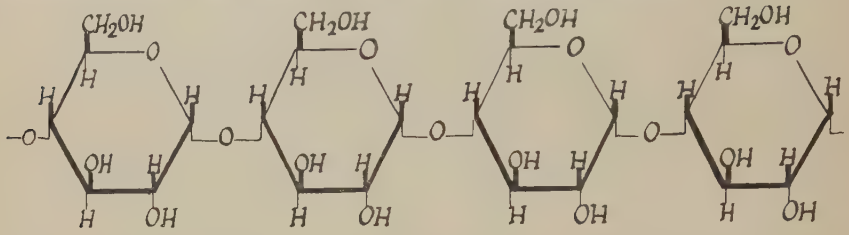
α-Maltose



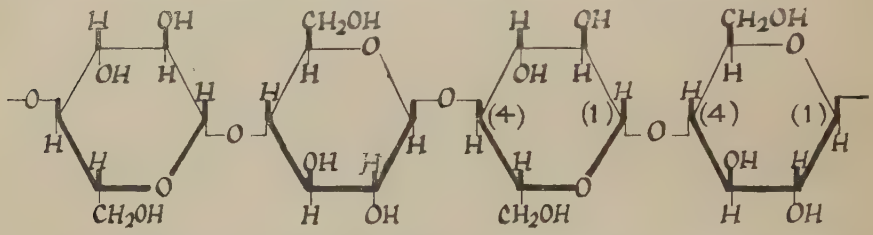
β-Cellobiose

Such evidence may well be fundamental to any plan for the allocation of constitutions to the two polysaccharides. Considered alone, it points definitely to the conception of structural formulæ in which conjugated maltose units comprise the starch molecule, and the analogous linking of cellobiose residues end to end represents the constitution of cellulose.

A.—Conjugated Residues of Maltose : (α-linking of glucose)



B.—Conjugated Residues of Cellobiose : (β-linking of glucose)



The exigencies of the structure and configuration of cellobiose necessitate that the glucosidic junction should be alternately below and above the plane of the rings as shown here diagrammatically. To attain this end the alternate rings must be rotated through 180°. (See Plate II.)

This is the simplest basis on which to formulate these carbohydrates. It is a basis which recognizes the significant facts of the hexagon structure of glucose units in maltose and cellobiose, and also the ease with which these disaccharides are generated from the two polysaccharides. It recognizes α-glucose as the essential residue in starch, and β-glucose as the corresponding residue in cellulose. Associated with this conception is the idea that these units are joined entirely by glucosidic linkings and the wide inference may be drawn that starch and cellulose are analogues of the simplest glucosides. On this view the optical rotation data should reveal a generic relationship, and the following figures are of interest :

	$[\alpha]_D$		$[\alpha]_D$
α-Methylglucoside	+ 159°	Tri-acetyl starch	+ 170°
Tetra-acetyl-α-methylglucoside	+ 131°	Tri-methyl starch	+ 208°
Tetramethyl-α-methylglucoside	+ 154°		
β-Methylglucoside	— 34°	Tri-acetyl cellulose	— 22°
Tetra-acetyl-β-methylglucoside	— 18°	Tri-methyl cellulose	— 18°
Tetramethyl-β-methylglucoside	— 17°		

If this evidence were considered sufficiently convincing there would remain little more to be decided than the number of such glucose units which go to constitute the complete molecule of starch and cellulose. The question arises : Are the continuous chains made up of a large number of maltose residues in starch, and of cellobiose residues in cellulose ? Assuming the number of such biose groups to be thirty or more, it is conceivable that the presence of one reducing hexose terminally situated in so long a chain would not reveal any reducing action. So large a structure need not display a constant or measurable molecular weight or conform to any prevailing ideas of a molecular unit limited in size. On the other hand, the suggestion emerges that the two ends of the long chain may unite in a large ring structure on the plan of the polymeride of the lactone figured on page 79, but having the chain made up of six-atom rings.

On this view all linkings will be glucosidic and all reducing groups will participate in the mutual union of neighbouring hexose residues. Both these conceptions arise from the mode of linking outlined in the above formulæ, and each may apply to a specific polysaccharide. On the latter interpretation the molecule would be limited in size although its magnitude may be too large for measurement by molecular weight methods.

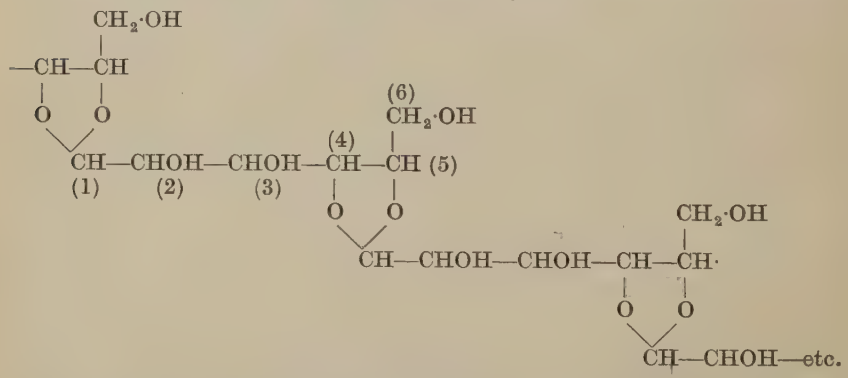
Starches are described as containing at least three constituents which can be separated : amylose, amylopectin, and hemicellulose. The hemicelluloses from wood contain carboxyl groups [1] formed from the side-chain or primary alcohol residue, and carbon dioxide may be eliminated without disturbance of the essential polysaccharide structure. This mechanism probably connects the starches or cellulose with the pentosans. It is claimed that amylose and amylopectin contain dissimilar kinds of linkings, the former being constructed on the plan of condensed α-glucose residues, whilst the latter is said to contain both α- and β-glucoses. The amylose portion furnishes only maltose on hydrolysis, whilst it is claimed that from amylopectin

[1] Candlin and Schryver, *Proc. Roy. Soc.*, 1928, **103**, 365 ; M. H. O'Dwyer, *Biochem. J.*, 1926, XX, 656.

a trisaccharide as well as maltose can be isolated. Potato starch is almost free from the hemicelluloses.

A. R. Ling has advanced a formula for amylose which represents a closed chain of six α-glucose residues.[1] Originally this was formulated on the basis of the older five-atom ring structure of glucose, and it was difficult to conceive of a reasonable arrangement in space of six closely packed residues on a geometrical plan. The determination of the hexagon structure for each of the glucose residues in maltose minimizes this difficulty, and a modified conception of this structural model is described on page 96.

The methylation of precipitated starch furnishes the trimethyl derivative in a yield of 89 per cent. whilst an almost quantitative conversion [2] of cellulose into trimethyl cellulose is also recorded. The products still display the properties of complex polysaccharides. These conversions are not the result of tedious and prolonged treatment of the carbohydrates with methylating agents, but are accomplished by no more than three to six treatments with the reagents. From the trimethyl starch and the trimethyl cellulose so prepared, an almost theoretical yield of the crystalline 2 : 3 : 6-trimethyl glucose is obtainable. It is therefore evident that probably the whole of the starch and the whole of the cellulose structures are built up on the principle that the groupings at positions 1, 4, 5 in the glucose unit are concerned in the internal structural arrangements, whilst at positions 2, 3, 6, there remain free hydroxyl groups. These facts are consistent with the view that starch and cellulose may be formulated in the manner represented on page 84.



One cannot evade the conclusion, however, that if the evidence just given be considered alone it affords no proof of the existence of glucopyranose rings in the polysaccharides, and indeed the above

[1] Ling and Nanji, *J.*, 1923, 2666.

[2] Haworth, Hirst and Webb, *J.*, 1928, 2681; Freudenberg and Braun, *Annalen*, 1928, **460**, 288. (*Compare* Denham, *J.*, 1921, 77; Irvine and Hirst, *J.*, 1923, 518; Irvine and Macdonald, *J.*, 1926, 1502.)

scheme originally suggested by Tollens [1] would equally explain these isolated facts (see previous page).

Unless we are to neglect the analogy to be drawn from the ascertained structure of maltose and cellobiose, then it seems most reasonable to postulate a ring form for each glucose unit in those polysaccharides which give rise to maltose and cellobiose. The proof of the six-atom ring form of glucose in each of these disaccharides has been outlined in a preceding chapter, and it seems at present the most hopeful basis on which to found any real conception of the constitution of the polysaccharides. It must, however, be admitted that, helpful as this analogy between disaccharides and polysaccharides may be, it is founded on a hypothesis which has not been proved. Chemical data which would enable a decision to be reached are lacking, although other experimental evidence in support of the six-atom ring in cellulose will now be outlined. If it were the case that maltose and cellobiose are not preformed in starch and cellulose, then there arise several interpretations of the existing data which may be near the truth. The presence of gluco-furanose (γ-glucose) residues in starch is a favourite hypothesis of some workers, the assumption being that maltose is a reversion product from an intermediate hydrolysis fragment of starch. Again, however, there is no satisfactory experimental basis either for the acceptance or rejection of this view.

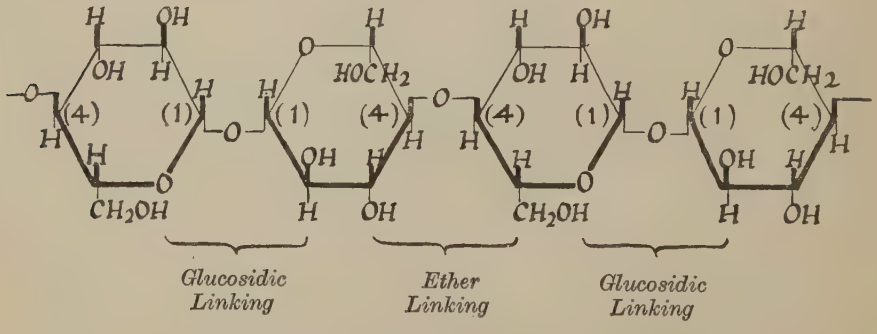
A welcome development, which promises a hopeful solution of some of the difficulties in this complex field, is the newer work on the determination of the crystal-lattice of cellulose. Sponsler and Dore [2] have now interpreted the X-ray diagram in the light of the new structural formula of glucose. They found that when the six-atom ring form of glucose is applied in their interpretations of the crystal structure of cellulose, it provides a most satisfactory explanation of the X-ray spacings. This conclusion may well be of great significance in the solution of many problems. These authors prefer to adopt the pyranose structure for the glucose units, to the exclusion of the older formula of the five-atom ring. Their work furnishes in a most interesting way a justification for the new formulæ which have been assigned to the sugars. From an examination of ramie fibres Sponsler and Dore have calculated the axes: $a = 10{\cdot}8$, $b = 12{\cdot}2$, $c = 10{\cdot}25$ in Ångstrom units for the crystallographic unit in cellulose. This unit cell accommodates eight glucose residues. The dimension along the fibre axis, indicated by $c = 10{\cdot}25$, represents exactly the length of two gluco-pyranose units in a chain, of which the units are mutually linked by the intermediary oxygen atom. The chains are said to

[1] *Handbuch der Kohlenhydrate* (Leipzig, 1914), p. 564.

[2] *Colloid Symposium Monograph*, New York, 1926, 174; Sponsler, *J. Gen. Physiol.*, **1926, 9, 677.**

be accommodated in rectangular spacings, 6·1 × 5·4 Å apart, and the component parts in each chain are repeated at intervals of 10·25 Å and contain two C_6 units. Within this elementary cell the atoms are so arranged that a number of planes occur at intermediate distances which bear a simple numerical relation to the 10·25 dimension. The length of the single unit of glucose is thus given as 5·15 Å, agreeing closely with the length of the atom model constructed of spheres having a diameter of 1·5 Å, the value which is determined from other X-ray data on hydrocarbons, the fatty acids, and the diamond. The corresponding length of a model constructed as a five-atom ring form of glucose is 5·4 Å and is thus too large. The ramie fibre is looked upon as a hollow cylinder ; in the walls of this the crystallographic units are distributed in such a manner that the diagonal of the 6·1 × 5·4 spacing always occupies a tangential position, and the width of the glucose unit is accommodated along this diagonal. These requirements are met by the adoption of a formula for cellulose which is similar to that outlined on page 84.

In the previous formula, however, the hexose units are mutually linked by glucosidic oxygen attached at carbon atoms 1 and 4 alternately throughout the chain. We have seen that this arrangement fulfils all the conditions required by the chemical properties of cellulose. A modification of this arrangement would be to suppose that the hexoses are mutually linked by oxygen atoms attached not alternately at 1 : 4 positions throughout, but at positions 1 : 1, 4 : 4, 1 : 1, etc. Sponsler and Dore claim that such a mode of attachment best explains the occurrence of the 3·4 Å spacing in their X-ray diagram. Their modified formula may be represented by the following scheme :



Whilst the general conclusion contributed by this work provided a remarkable confirmation of the thesis which has been developed in the preceding pages of this book, yet the precise form which the authors have adopted in their picture of cellulose raises certain diffi-

culties. They suggest that the mutual union of the hexagon form of glucose occurs alternately through *glucosidic* and *ether* linkings, and that cellobiose is not to be regarded as preformed in cellulose, but as a reversion product. The ether type of linking which these authors adopt for the union of the glucose units, through the hydroxyls at the 4 : 4 positions, has not been encountered experimentally in the sugar series. It represents a grouping which may be expected to offer resistance to the usual hydrolytic agents, and for this reason it is hardly likely that it would have escaped detection in the products of hydrolysis. Efforts will doubtless be made to obtain experimental evidence bearing on this view, but it can scarcely be accepted without reserve. Most of the properties of cellulose can, however, be explained on the basis of this X-ray evidence.

More recently other workers have attempted a new interpretation of the X-ray data from ramie cellulose. K. H. Meyer and Mark [1] appear to have calculated a different crystallographic cell having the dimensions 8·7, 7·9, 10·3 ; the last dimension represents the length along the fibre axis, and this only is in agreement with the figures of Sponsler and Dore. It is difficult to reconcile these two sets of measurements. Meyer and Mark suggest that their unit cell accommodates six glucose units as compared with the eight units of the previous authors. In general, however, they adopt the same view, namely, that along the fibre axis the hexagonal form of glucose is linked through intermediary oxygen atoms. But they regard the linking as being invariably of the glucosidic type, consisting in the repetition of cellobiose units throughout the length of the chain. They appear to rely mainly for their X-ray data on earlier determinations of other workers.

Chemical and X-ray examinations of the polyoxymethylenes by Staudinger [2] have furnished most important evidence concerning the relation between the content of the unit cell as revealed by X-ray methods, and the nature of the structural unit. These long chain compounds have unit cell structures which contain a very small number of (CH_2O) units whereas there is definite chemical proof of their high molecular weight. These results must obviously be kept in mind when considering any interpretation of the X-ray data for cellulose and other polysaccharides. The size of the structural unit in cellulose, for example, cannot be determined on the basis of X-ray data alone, and, as Staudinger has recently pointed out, the necessary chemical evidence which would enable this to be done is at present entirely lacking.

[1] *Ber.*, 1928, **61**, 593 ; K. H. Meyer, *Zeitsch. angew. Chem.*, 1928, No. **34**, 935.
[2] *Ber.*, 1926, **59**, 3025 ; *Ber.*, 1928, **61**, 2427.

Conformation of Models.—Current theories in regard to the formulation of strainless rings may penetrate in the future to the group of the polysaccharides. At present there is insufficient experimental evidence available for any kind of opinion on this problem. In view of the possible importance of these ideas and of the tentative suggestions of two investigators, the possibilities of the construction of atom models on such a principle have been explored, and a few of the conclusions are appended.

The cyclic group comprising five carbon atoms and one oxygen atom may have the centres of all the atoms co-planar. In this event some strain is introduced at the valency bonds of the atom model if this be constructed on the plan of allowing the tetrahedral angle between each valency bond. The diameter of the carbon atom is given as 1·55 for aliphatic compounds and for the diamond. The diameter of the oxygen atom is probably very nearly the same as carbon. It has not been expressly determined for the grouping C—O—C ; but in the grouping — C = O the value is given as 1·35.

Models constructed by using spheres of these dimensions are of the greatest possible value in the study of complex sugars and carbohydrates.

By adopting an arrangement of the six-atom ring which is not co-planar almost the whole of the strain is eliminated. A form in which only the oxygen centre is outside the general plane of the carbon centres is shown in the sketch Fig. I,

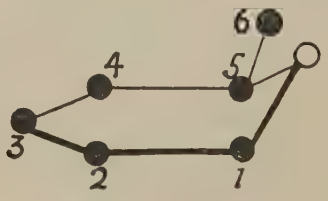


Fig. I. Perspective.

Fig. I. Elevation.

but this is merely a special case of the general one which is now to be considered. Sponsler and Dore have selected the zigzag arrangement of all the atoms in the glucose ring, and here there are two possible alternatives, the atomic centres being indicated in the sketches on the next page (Figs. II and III).

These skeleton models are mirror images of each other, and in

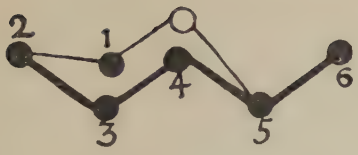


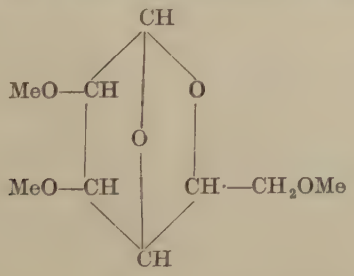
Fig. II. Perspective.

Fig. III. Perspective.

Fig. II. Elevation.

Fig. III. Elevation.

each there are two ways of arranging the H and OH groups. These furnish two models for *d*-glucose and two for *l*-glucose. The second of these figures gives the more regular arrangement for the *d*-series, since the configuration of *d*-glucose in this model allows of the OH at C_2 being in the same plane as C_3, and the OH at C_3 in the same plane as C_2 and C_4, so that the whole of the carbon and oxygen atoms in this *d*-glucose model are accommodated in two planes only. Such a choice can only be an arbitrary one, but the selection of this model facilitates the closest possible packing for *β*-glucose units, though not for *α*-glucose. A new aspect of stereoisomerism arises, however, if these assumptions be made, in that there are evidently mirror image forms of the ring, and this produces an enantiomorphism quite independently of the asymmetrical arrangements of the addenda of the ring. The above models represent *trans* forms constituted on the Sachse principle. Besides these, six *cis*-models can be constructed of the Sachse type for *d*-glucose, making a total of sixteen possible arrangements for *d*- and *l*-glucose. One of the cis-types is evidently possible since trimethyl 1 : 4-anhydrogluco-pyranose has been prepared,[1] and this has the same bridged ring as in camphor.



[1] Freudenberg and Braun, *Annalen*, 1928, **460**, 288.

These considerations open up a large field of inquiry into the conformation of groups as distinct from structure or configuration, but they cannot adequately be dealt with here.[1]

By adopting the above *trans*-conformation of *d*-glucose (model III) the mutual linking of two *β*-glucose models at the 1 : 4-positions is greatly facilitated. The co-planar model seems to require that each alternate ring in a chain of *β*-glucose units should be revolved 180° through the vertical plane (see page 84) before the linking can occur, since the 4 and 1 hydroxyls are on opposite sides of the ring. The *trans* model requires only that each ring should turn in its own plane through 30° to achieve the same result. If cellulose is constituted on the basis of cellobiose the latter rotational movement would seem the simpler and more rational mechanism. In plant synthesis it is probable that each glucose unit is presented to its neighbour in a form in which it can easily combine. It is difficult to imagine a selective mechanism in which each alternate unit is required to revolve through 180° before mutual linking occurs.

The conformation of the model attained on this principle of linking is Fig. IV, which is seen to preserve the numbering of the constituent atoms in the clockwise direction only, instead of having alternate pyranose units numbered counter-clockwise as in the formula B on page 84 of which the skeleton model is shown in Fig. V.

This type of diagram is used deliberately here in order to express with clearness the co-valency bonds in each linking. It represents the space model *in projection* and in skeleton form. The models actually used to illustrate these and other possible structures should preferably be those in which the spheres are in contact through hidden bonds, since only by this means can the relationships of the structures be fully comprehended. In the above projection Diagram IV, the angle between adjoining atoms appears as 120°, whereas in the actual space model the angle is 109° 28′. Such models of IV and V are given in the plate at the end of this chapter.

A point to be noticed from the Diagram IV (and also by *handling* the models) is that interstitial hexagon spaces occur between each pair of gluco-pyranose units, and that other such hexagonal groupings are developed from the addenda. The model assumes a more compact form than V, and when viewed edgewise (or in elevation) the single line of groupings resembles the zigzag conformation which has been allocated, on the basis of X-ray data, to the hydrocarbons and fatty acids. Assuming that oxygen, when consorting with tetrahedral carbon in a six-atom ring, has approximately the diameter of the carbon atom, it may be expected that a molecular structure

[1] Haworth and Hirst, *J.*, 1928, 1221.

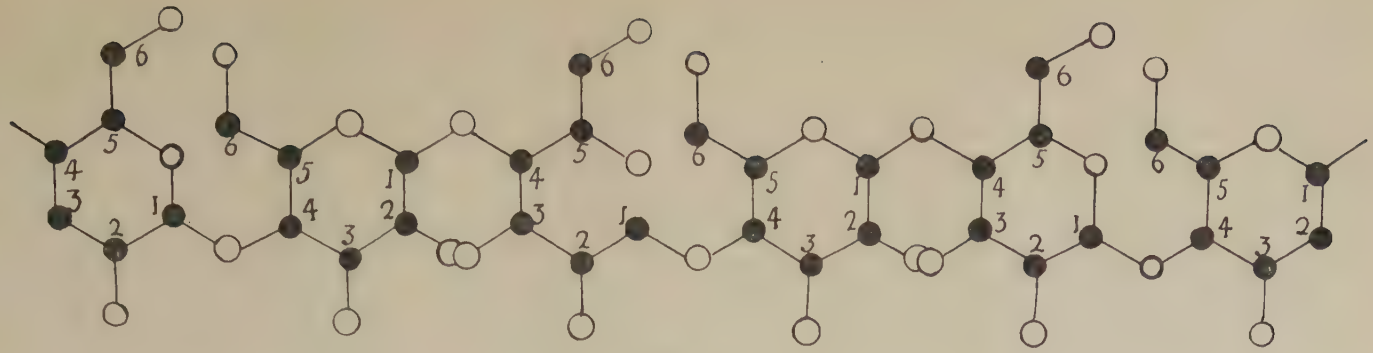


FIG. IV.

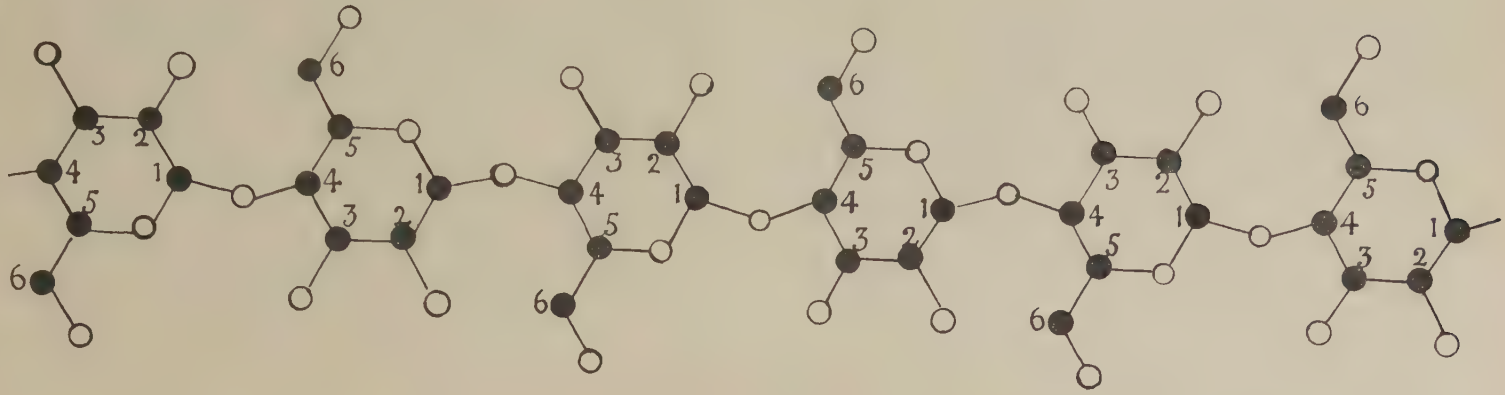


FIG. V.

built up in accordance with the principle of this model would give X-ray spacings corresponding to 2·57 Å, which is the distance between centres of three adjoining carbon atoms in hydrocarbons. Four such spacings would represent the total length of a cellobiose residue (= 10·28), and would not be far removed from the value actually calculated for cellulose (10·25).

A characteristic feature of the type of linking shown in IV is that, allowing the tetrahedral angle to the glucosidic oxygen atom, the junction of successive pyranose units need not necessarily proceed by formation of a chain which is straight, but the formation of a curved chain is equally permissible without introducing strain or alteration of the tetrahedral angle between any two co-valency bonds. This principle admits of the construction of models of looped chains or large cyclic structures which represent complete molecular units of any dimensions from $(C_6H_{10}O_5)_6$ upwards, increasing by four units (or a multiple of four) to $(C_6H_{10}O_5)_x$. Inasmuch as this possibility is to be applied in subsequent paragraphs dealing with the linking of α-glucose units, it need not be developed separately here. It is sufficient to indicate that the *trans* form of strainless rings which are under discussion for β-glucose are equally adaptable for the large cyclic conformations now to be considered for α-glucose as a strainless *cis*-hexagon.

These models by no means exhaust the number of possible arrangements or conformations for β-glucose. Other and obvious alternatives have been studied (see plate at end of this chapter). But the principle underlying the construction of models of mutually linked gluco-pyranose units may have been made sufficiently clear by the exposition which has been attempted. It is intended to be suggestive, and therefore to lead to experimental schemes of work which may prove fertile.

For the α-glucose unit, which is probably present in starch, none of the models previously considered admits of the alignment of consecutive hexagons in the same horizontal plane, except by altering the tetrahedral angle of the valencies of the glucosidic oxygen. Owing to the OH groups at C_1 and C_4 being on the same side of the figure, the only geometrical arrangement which is at all feasible (if the tetrahedral glucosidic oxygen is to be retained) is to place consecutive rings parallel to each other. This is a conceivable structure for starch, but other kinds of conformation are available. One of these is a strainless *cis*-ring in which the hexagon model of glucose is folded along the carbon atoms 2 and 5, a conformation which brings the 2, 5, and 6 carbons into line in the same plane and the 0, 1, 3, 4 atoms in a second plane. The hydroxyls at C_1 and C_4 then extend outwards and the mutual linking of pyranose residues of the α-glucosidic

type furnishes an almost flat structural model in perfect alignment. The projection (Fig. VI), although not the three-dimensional model

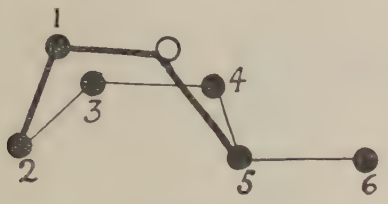


Fig. VI.

itself, gives the same arrangement as that shown in Fig. IV above. Manipulation of this model by a rotation at the glucosidic oxygen bonds at (*a*) provides a means of changing the direction of the chain through an angle of 120° in its own plane (Fig. VII).

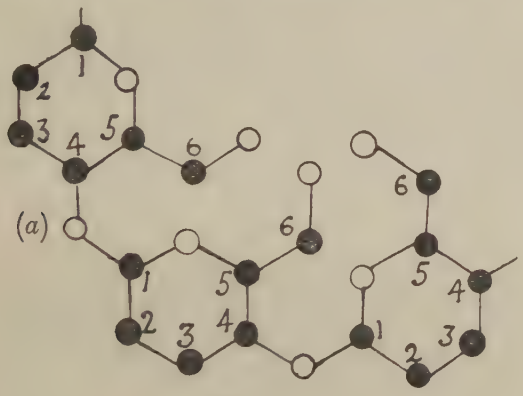


Fig. VII.

If six *α*-glucose models are joined on this plan the combined figure is also hexagonal and has a remarkable pattern. Although only six glucose models are involved, the whole is a geometric figure of nineteen hexagonal spacings (Fig. VIII). Whilst in projection the angles between adjoining bonds are 120°, in the three-dimensional model they are actually the tetrahedral angles of 109° 28′.

The formula ascribed by Professor A. R. Ling [1] to a constituent of starch, having $(C_6H_{10}O_5)_6$ as the molecular unit, is thus capable of formulation in this remarkable way. Moreover, a series of such models placed symmetrically in space would leave practically no interstices between molecular units and would present a uniform hexagonal pattern. This is facilitated by the fact that whilst the OH groups at C_6 in each unit lie in the centre of the figure and in the

[1] See page 86.

same plane, the OH groups at C_2 and C_3 lie at the periphery and extend above and below the general plane.

Whether such a model will receive experimental sanction is a problem for the future. But any geometrical arrangement for α-glucose units which is ultimately adopted must satisfy the valency conditions of an appropriate model. Here is one only of several which can be devised, and such a means of visualizing possible structural conformations may be of value in directing future experimental study into promising channels.

It is not suggested by these speculations that free or unimolecular

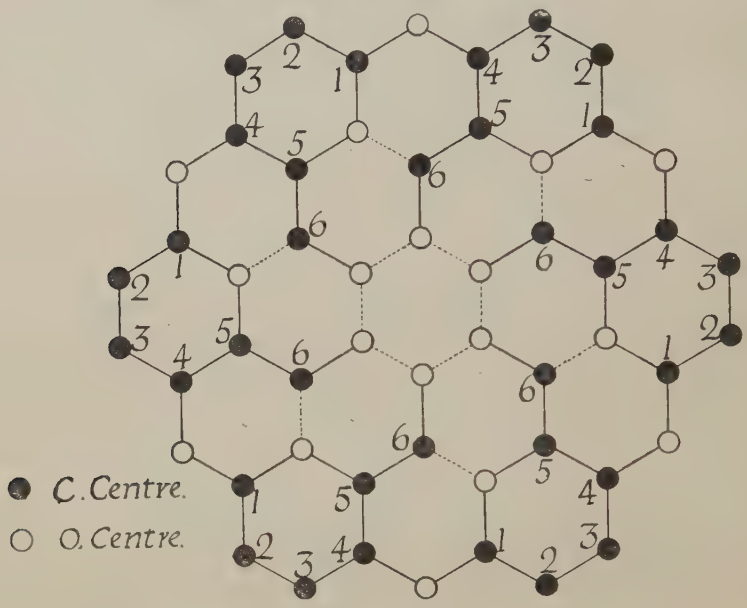
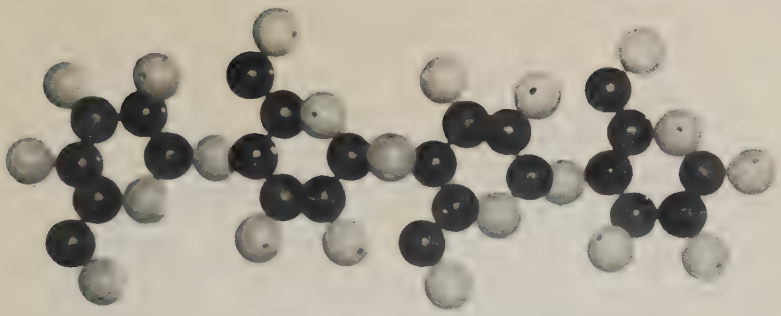


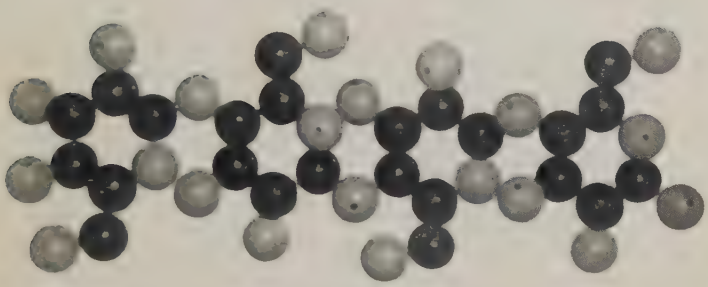
Fig. VIII.

glucose exists in more than the recognized forms. But glucose is usually a product of hydrolysis from some more complex molecule. In combination with similar units the glucose residue may tend to assume that ring conformation which will facilitate that state of combination and at the same time will conserve space.

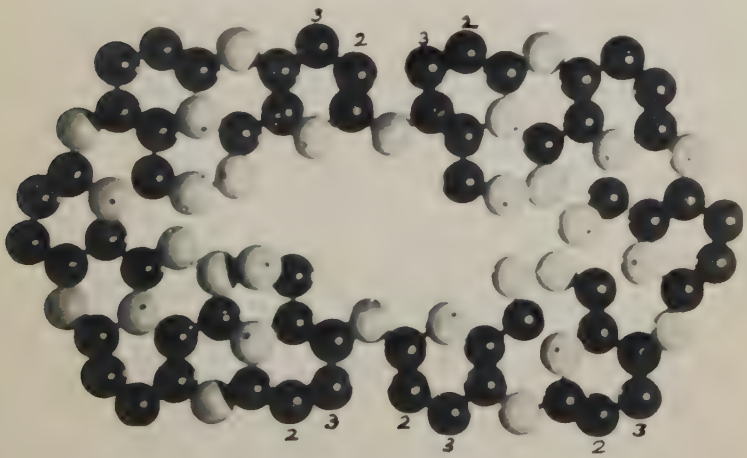
A far more extended study of the chemistry of cellulose and starch is imperative if a final solution to these problems is to be attained. The chief object of this closing chapter, in so far as it must be speculative, is to provoke discussion and to stimulate endeavour.



MODEL OF FIG. V



MODEL OF FIG. V ROTATED ROUND AXES OF GLUCOSIDIC OXYGENS



MODEL OF TEN α-GLUCOSE UNITS ARRANGED AS IN FIGS. VII AND VIII. FOR CLEARNESS, O-ATOMS OF OH-GROUPS AT POSITIONS 2 AND 3 ARE OMITTED. β-GLUCOSE UNITS CAN BE ARRANGED SIMILARLY (SEE TEXT, PAGE 95)

CONFORMATIONS OF COMPLEX MODELS

INDEX OF AUTHORS

SUBJECT INDEX